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A CLINICAL METHOD FOR STUDYING TITRATABLE ALKALINITY OF THE BLOOD AND ITS APPLICATION TO ACIDOSIS.

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In the investigation of the extremely varied properties and functions of the blood considerable attention has been directed toward the chemical analysis of its fundamental constituents. Satisfactory methods have been developed for the qualitative and quantitative estimation, not only of many of the common constituents, but also for some of the substances which occur in relatively minute amounts. However, the determination of one of the fundamental characteristics of the blood, namely, its chemical reaction, is very unsatisfactory. A variety of distinctly different principles have been utilized in the development of methods for studying the reaction of the blood. These methods, however, have not only failed to afford the information which is required clinically, but they are also unsuitable from an experimental standpoint.

Inasmuch as the expression, "the reaction of the blood," has come to be used in two senses, a brief discussion of term is advisable. In its strict sense, the reaction of the blood depends upon the physico-chemical balance between the hydrogen and hydroxyl ions which it contains. In this sense the blood is hardly more alkaline than distilled water. More loosely, the reaction of the blood was formerly stated in terms of its behavior toward indicators and its ability to neutralize acids or bases. Although normal blood serum reacts as an acid toward a few indicators, notably phenolphthalein, yet it is definitely alkaline toward the majority of the common indicators and, since the alkaline reaction to these indicators can be maintained even after the addition of small amounts of acid, the blood is commonly spoken of as an alkaline fluid. From these considerations it will be seen that, although the physico-chemical measurements show the hydroxyl ion content of the blood to be very low, yet the blood contains a moderate quantity of substances which can readily yield hydroxyl ions upon the introduction of the hydrogen ion of an acid. Thus it is seen that two distinct interpretations have arisen for the term "reaction of the blood." In the present paper this term will be used in its physico-chemical sense to indicate the balance between hydrogen and hydroxyl ions and the behavior of the blood with indicators toward acids and bases will be designated as the titratable alkalinity. The question at once arises as to the relative importance in biological work of these two factors, namely, (1) the hydroxyl ion content of the blood, and (2) its titratable alkalinity. It has been shown by Henderson [1]

[1] Henderson: Am. Jour. Physiol., 1908, XXI, 427. See also Palmer and Henderson, Arch. Int. Med., 1913, XII, 153.

that the hydroxyl ion content of the blood varies but slightly, even under extreme pathologic conditions. This relatively constant value of the hydroxyl ion concentration is maintained, in part at least, at the expense of the titratable alkalinity. However, the detection of changes in the titratable alkalinity is difficult and unsatisfactory. The purpose of the present paper is to consider a method for detecting such changes and its application to clinical conditions, especially to acidosis. There are a variety of conditions in which the possibility of an acidosis has been suggested, more especially in certain of the nephropathies and anæmias, in eclampsia, and other complications of pregnancy and in several of the diseases of children, as in rickets and in the gastrointestinal disturbances.[2] Instead of examining for certain specific acids in each of these conditions, it would be much more satisfactory if a method of general application could be evolved which would not require the isolation and recognition of the acid in question. The determination of the tolerance to sodium bicarbonate affords such a method with the reservation, however, that the effect of renal lesions on the validity of this procedure has not been determined. Any methods for the detection of acidosis which depend upon the examination of the urine, may readily be inapplicable in the presence of renal disease, and, unfortunately, many of the conditions in which acidosis is suspected are complicated by disturbances of renal function.

Of the methods which have been proposed for determining the titratable alkalinity we need not consider the indirect procedures, such as the determination of the carbon dioxide content or the changes in the spectrum upon the addition of acid. Direct titration with some of the ordinary indicators has given the most suggestive results. As a type of this method we may consider the titration of blood serum against a standard acid with one of the azo dyes as an indicator. This group of indicators is extremely sensitive to alkalies, and apparently the underlying object in the use of them is to measure the full value of the titratable alkalinity. One of the principal objections to the procedure is that the end-point is extremely unsatisfactory. The protein of the serum is largely responsible for the interference with the end-point, but the removal of the protein would remove one of the important sources of titratable alkalinity. Furthermore, the maximal differences that are observed between normal and pathologic sera are without any striking significance. This necessitates the measurement, under unfavorable conditions, of small differences in the quantity of acid required for neutralization.

To obviate the difficulties of the titration methods it would seem desirable to develop a purely qualitative method in which all normal sera would react distinctly alkaline, whereas the pathologic sera would be distinctly acid to a given indicator. In the attempt to obtain such a result, the following departures from the usual titration methods were considered:

1. The selection of an indicator to which the serum of all normal individuals is acid, depending upon the conversion of the acid carbonates of the blood to normal carbonates in order to obtain an alkaline reaction in normal individuals. The variation in the reaction of the blood toward different indicators is due, principally, to the sodium bicarbonate which it contains. This salt reacts as an acid to certain indicators, such as phenolphthalein, and as a base to others, notably methyl orange. However, on boiling, carbon dioxide is driven off and the normal carbonate is formed. The latter hydrolyzes in water and reacts as a base to all indicators. Thus, the content of sodium bicarbonate in normal blood is sufficient to explain why the serum reacts as an acid to phenolphthalein, but, after diluting with water and boiling, it will react sharply alkaline.

2. The removal of the interfering protein from the serum with the purpose of determining definite changes in the titratable alkalinity rather than the measurement of the total titratable alkalinity.

3. The substitution of another solvent for water to reduce the amount of hydrolysis and ionization. Before sodium carbonate can react with phenolphthalein, it must ionize and hydrolyze. These changes take place very readily in water, but by substituting some other solvent it might be possible to reduce these changes to such an extent that a trace of carbonate would give no reaction, whereas the amounts normally present in the serum would give a pronounced reaction.

From these considerations it is conceivable that conditions might be obtained in which all normal blood would be distinctly alkaline, whereas pathologic blood in which a definite diminution of sodium bicarbonate had occurred, might be distinctly acid under the same conditions. The basis of such a test would be essentially qualitative rather than quantitative. The feasibility of such a procedure has been tested in the following ways, namely:

1. Experiments with serum in vitro.
2. Experimental production of acidosis in lower animals.
3. Investigation of clinical conditions.

Selection and Preparation of an Indicator.—There have recently come into use a large number of indicators whose end-points change at varying concentrations of hydrogen and hydroxyl ions. Phenolphthalein is one of the most convenient of those which change to an acid reaction at a comparatively low concentration of hydrogen ions. The ordinary diacid preparation was employed for all qualitative work, using a ½% solution in absolute alcohol. Later, for quantitative measurements, sufficient alkali was added to give a faint tinge of color to a large bulk of solution with the expectation that the colorless monosodium salt would change more readily to the red disodium salt than the acid itself. As a routine, 3 drops of either solution were employed; this indicator is such an extremely weak acid that accurate measurement proved to be unnecessary.

Removal of Protein and Selection of Solvent.—The removal of protein and the substitution of another solvent for water was combined in one step by precipitating the serum with alcohol. The removal of the protein avoids any interference with the end-point and at the same time removes a definite fraction of alkali-yielding substances which are probably fairly persistent, thus affording the possibility of obtaining a more

[2] See Ewing: Arch. Int. Med., 1908, II, 330.

sensitive test than could be secured with the aqueous dilutions of the whole serum. The removal of all traces of protein was not attempted, but practically complete removal was obtained by precipitation with absolute ethyl alcohol.

Experiments in Vitro.—The purpose of the tests in vitro was to determine whether the addition of minimal quantities of acid to serum could be detected by the use of phenolphthalein under conditions in which the titrations by the usual methods would not reveal the addition of the acid. To 1 cc. quantities of normal human serum increasing quantities of standard sulphuric acid were added. With the lower quantities of acid, sufficient water was added to make a final volume of 2 cc. of the mixture of acid and serum. The following observations were made on these specimens:

1. Reaction of the residue after incineration in platinum.
2. The reaction after diluting with water and boiling.
3. Precipitation of the protein with alcohol and the reaction of the alcoholic filtrate.

For the incineration in platinum the specimens were ashed at dull red heat. The residue was dissolved in water and the reaction tested to phenolphthalein. For the aqueous tests the mixture of serum and acid was diluted with about five parts of water and, after boiling freely for a minute, the reaction to phenolphthalein was tested. For the tests after removal of the proteins, 2 cc. quantities of the mixture of acid and serum were added, drop by drop, to 25 parts of absolute ethyl alcohol. The mixture was then shaken thoroughly and filtered. The filtrate, after the addition of phenolphthalein, and without any washing of the precipitate, was evaporated to dryness on a water bath.

The examination of a few specimens was sufficient to determine the characteristics of the reactions which were obtained under these conditions. The reactions with normal sera were essentially similar to the behavior of sodium hydroxide with phenolphthalein under the same conditions. The alcoholic filtrate upon concentration on a steam bath, rapidly took on a deep red color. This color was dependent upon the temperature of the solution, for upon rapidly cooling the solution the color faded markedly or disappeared completely. This change took place immediately before there was time for the absorption of carbon dioxide from the air, and was due apparently to a diminished dissociation with the lowering of the temperature. Upon evaporation to dryness the residue retained its red color for many hours. As a rule, six to eight hours heating on the steam bath were required to decolorize the residue. As the quantity of acid was increased, the residue, though red at first, rapidly became colorless on heating. With still further addition of acid, no color appeared at any stage of the evaporation, but the addition of water to the residue produced a red color. Lastly, those cases occurred in which no color was obtained during the evaporation or upon the addition of water to the residue. In some of these cases, however, the boiled aqueous dilution of the serum would give a distinct color with phenolphthalein.

Pure sodium hydroxide gave reactions with phenolphthalein which corresponded to the behavior of normal serum. Traces of sodium hydroxide (0.5 to 0.1 cc. of N/200 solution) when added to 25 cc. of absolute alcohol gave no color upon evaporation to dryness. The residue upon dissolving in water became distinctly pink or red. The residue, after the evaporation of phenolphthalein with an aqueous solution of sodium hydroxide, retained its red color, often for as long as twenty-four hours on a steam bath. On heating in an air bath at 90° C. the pink color was almost completely discharged in two or three hours.

The results of the reactions with phenolphthalein have also been compared with the usual titration methods for blood serum. Of the various procedures, the use of standard acid, with one of the azo dyes, was selected since this is the most feasible for clinical purposes, and in the hands of some investigators it has given rather suggestive results. Many technical variations have been suggested in the application of this procedure. Of the azo dyes, dimethylamidoazobenzene (Töpfer's reagent) is recommended by Strouse.[3] The principal advantage of the technique devised by Wright[4] for the quantitative titration of serum with acids appears to lie in minimizing the quantity of serum which is required. Inasmuch as moderate quantities of serum were avilable in the present work, the titrations were carried out in the ordinary manner. The following routine applies to all the results with Töpfer's reagent which are recorded in this paper. One cubic centimeter of serum was diluted with water until the color of the serum was practically eliminated; usually about 100 to 200 cc. of water were required; 3 to 4 drops of a ½% solution of the indicator in 95% alcohol were used. The addition of N/10 hydrochloric acid was continued until the distinct yellow of the Töpfer's reagent, not only had deepened considerably in color, but until a definite pink shade appeared. So much acid was required to effect this change that there was no advantage in using a higher dilution than N/10. Indeed, in some instances, no end-point was obtained, but the addition of a relatively large proportion of acid effected only a very gradual change in color through all shades from yellow to red. It is understood, therefore, that the figures for Töpfer's reagent represent only approximations and for the most part do not represent a sharp end-point.

From these data (Table I) it is evident that the quantity of acid which is sufficient to produce a definite change in the reaction to phenolphthalein is much too small to be detected with any certainty in the titrations against Töpfer's reagent under corresponding conditions. Thus the end-point with Töpfer's reagent can hardly be determined to greater accuracy than 0.1 or 0.2 cc. when a N/10 solution is used. However, the addition of the equivalent of 0.1 to 0.2 cc. of N/10 acid to 1 cc. of serum could be detected very readily by the use of phenolphthalein.

Experimental Acidosis.—In the application of these tests to conditions occurring in the animal body, it is to be expected that the results would depend largely upon the compensatory processes of metabolism. The efficacy of these processes might vary widely; therefore, the reaction was first studied in ani-

[3] **Strouse: Johns Hopkins Hosp. Bull., 1908, XIX, 137.**
[4] **Wright: Lancet, London, 1897, II, 719.**

mals in order that it might be tested under conditions in which a simple definite acidosis of known etiology could be established. For this purpose acidosis was produced by the administration of mineral acid. Among the various possibilities of this experimental acidosis the two extreme effects that might be produced are: (1) the carbonates in the blood may diminish under the administration of acid, or (2) such a condition may be incompatible with life, a fatal effect resulting before the carbonates are appreciably reduced.

TABLE I.

ADDITION OF ACID TO SERUM IN VITRO.

cc N/25 acid per cc. serum.	Behavior toward phenolphthalein. After removal of protein. In alcoholic solution.	Residue after evaporation.	In aqueous solution.	1-10 dilution of whole serum in water.	Incinerated residue in water.
FIRST SPECIMEN.					
None	Red	Red 7 hrs.	Red	Red	Red
0.05	Red	Red 1 hr.	....	Red	....
0.10	Red	Red 15 min.	Red	Red	....
0.15	Red	Red 15 min.	Red	Red	....
0.20	Red	Red 15 min.	Red	Red	....
0.25	No color	Red 5 min.	Red	Pink	Red
0.5	No color	No color	No color	Trace	Red
SECOND SPECIMEN.					
None	Red	Red 6 hrs.	Red	Red	....
0.25	Red	Red 15 min.	Red	Pink	....
0.4	Red	Red 5 min.	Red	Red	....
0.45	Red	No color	Red	Pink	....
0.5	No color	No color	Pink	Pink	....
0.6	No color	No color	No color	No color	Red
0.7	No color	No color	No color	No color	Red
0.75	No color	No color	No color	No color	Red
1.0	No color	No color	No color	No color	Red
1.5	....	....	....	No color	Red
THIRD SPECIMEN.					
0.25	Red	Red	Red	Red	....
0.5	No color	No color	No color	Pink	....
0.75	No color	No color	No color	No color	....
FOURTH SPECIMEN.					
0.25	Red	Red	Red	Red	....
0.5	No color	No color	Trace	Red	....
0.75	No color	No color	No color	No color	....

In case the carbonates do not diminish in the blood under the administration of acid, the question would arise as to the source of supply which could furnish sufficient carbonates or other alkali-yielding substances to meet the relatively large amounts of acid which it is possible to introduce into the body. It is readily conceivable that the blood might draw on the other tissues of the body for carbonates and death might result from the exhaustion of these tissues before the blood itself was markedly depleted. On the other hand, in case a definite reduction in the bicarbonate content of the blood takes place, there are a number of compensatory mechanisms by which the respiratory processes may be continued.

Some experiments were undertaken to determine whether, upon the introduction of acid into the body, sufficient change takes place in the blood serum to give it a neutral or acid reaction to phenolphthalein. Hydrochloric acid was administered through a stomach tube to rabbits after the manner of the original experiments of Walther.[5] A 10% solution in water of absolute hydrochloric acid was kept as a stock solution and this was diluted to ½% before use. Walther estimated that 0.9 gm. of hydrochloric acid per kilo per day was fatal for rabbits (approximately 25 cc. of a normal solution). For the production of an acute acidosis, I have used quantities of 0.5 gm. per day. In some experiments this was divided into two doses, one of which was given in the morning and the other in the afternoon.

For the examination of the serum with phenolphthalein the following routine was used throughout the remainder of the experiments described in this paper. The description is given in some detail, inasmuch as there are a number of minor factors which might definitely affect the results.

The serum was usually examined at the time of collection, especially in the important cases. In some instances it was stored in the refrigerator, but the examinations were always made within twenty-four hours after the time of collection. Absolute alcohol was chosen in preference to 95%, partly on account of its freedom from organic acids and also to reduce the amount of water in the reaction mixture to a minimum. In order to test its neutrality 0.1 cc. of N/200 sodium hydroxide was added to 25 cc. of alcohol, using 2 to 3 drops of phenolphthalein as indicator. The mixture was evaporated to dryness and the residue, on taking up in a few drops of water, gave a distinct color. For the incinerated specimens, a drop of serum was ashed at dull red heat in a platinum crucible. The residue was taken up in water and a few drops of phenolphthalein were added. The preparations with water were made in 1-10 dilution and boiled in a test tube over the free flame for one minute or more. In the case of normal sera a pink color appeared immediately upon the addition of phenolphthalein. In other cases it frequently happened that preparations which were colorless at first would develop a trace of color on standing for five to ten minutes. Such a behavior was somewhat unexpected, since phenolphthalein in aqueous solutions fades on cooling, owing to the absorption of carbon dioxide from the air. However, the preparations of normal serum in water also faded after several hours at room temperature.

High dilutions of normal sera in water, even 1 to 1000, usually gave a distinct color with phenolphthalein after boiling. As a rule, however, 1 part of serum was added to 10 parts of water. At this dilution, comparatively little precipitation of protein takes place on account of the alkali which is present. With a slight excess of acid, the solution remained compara-

[5] Walther: Arch. f. exper. Path. u. Pharmakol., 1877, VII, 148.

tively clear on heating, but after approximate neutralization by small quantities of acid, abundant precipitation of protein occurred on heating on account of the lack of alkali for the formation of the soluble alkali albuminate. This precipitate occasionally interfered with the detection of faint traces of color, since a practically colorless solution would sometimes become faintly pink upon high dilution; the results could hardly be misleading, however, as the interference is so slight and the source of this interference depends upon the absence of alkali.

For the preparations with alcohol, 1 cc. of serum was added, drop by drop, to 25 cc. of absolute ethyl alcohol. The mixture was then shaken thoroughly and filtered into an evaporating dish. Perfectly dry apparatus, or apparatus washed with absolute alcohol, was used throughout in order to exclude all water from the mixture except that which is contained in the blood serum. Without washing the precipitate, 3 or 4 drops of phenolphthalein were added to the filtrate and evaporation to dryness was carried out on a steam bath (not over 100° C.). Only a minimum amount of steam was used during the evaporation in order to avoid, as far as possible, the excessive absorption of water vapor by the alcohol. The method of recording the results requires careful explanation. In the preparation of the experimental mixtures, many gradations of titratable alkalinity were produced. The variations in the behavior of phenolphthalein with bases in aqueous and in alcoholic solution fortunately offers the possibility of detecting some of the gradations. The alcoholic filtrate at the moment of going to dryness approaches an aqueous solution on account of the water added with the serum. In many cases the alcoholic solution remained colorless till this point was reached. In these cases the residue, however, instead of retaining its red color on the water bath for several hours, usually lost it within five to fifteen minutes.

In a series of examinations, where a great variety of transitional stages occur, it is obvious that there will be considerable variation in the minor details of the results that are obtained. However, it has not seemed necessary or advisable to attempt to describe all of these details; only the more significant changes have been noted. For many reasons it is not desirable to attempt to record shades of color. However, it is obviously necessary to distinguish between cases in which a personal equation does not come into consideration, as for example, in those cases where a pronounced red or a barely discernible trace of pink is obtained. In compiling the results in tabular form, the term "no color" is used. This refers only to the shades of red and pink. In many of these instances the dry residue was tinged with yellow, presumably on account of the lipoids of the serum. The absence of any pink color with phenolphthalein means, of course, that the solution was neutral or acid. Some of the most pronounced cases were titrated with N/200 sodium hydroxide. However, there was not a distinct difference between the amounts of hydroxide required for the test specimens and for a blank control. No further attempt was made to measure any acid which may have been present. It is not essential for the interpretation of the results in the following tables, whether these colorless specimens are considered as neutral or faintly acid to phenolphthalein.

TABLE II.

EXPERIMENTAL ACIDOSIS IN RABBITS.

RABBIT I.

Day of experiment.	Grams HCl per kilo.	Interval between administration of acid and collection of blood.	Blood serum.				
			Alkalinity to Töpfer's in % N/1 solution.	Behavior toward phenolphthalein.			
				After removal of protein.			1-10 dilution of whole serum in water.
				In alcoholic solution.	Residue after evaporation.	In aqueous solution.	
1	None	....	0.7	Pink	Pink	....	Pink
	0.5	22 hrs.	0.7	No color	No color	No color	No color

RABBIT II.

1	None	....	0.8	No color	No color	Red	Red
	0.5	20 hrs.	0.7	No color	No color	No color	Pink

RABBIT III.

1	None	....	0.8	Red	Red 7 hrs.	....	Red
	0.5	20 hrs.	0.8	No color	No color	No color	Pink
2	0.5	26 hrs.	0.65	No color	No color	No color	No color

RABBIT IV.

1	None	....	0.7	Red	Red	....	Red
	0.25	6 hrs.	....	Red	Red	....	Red
	0.25	18 hrs.	....	Red	Red	....	Red
2	0.25	6 hrs.	0.75	Pink	Red	....	Red
	0.25	20 hrs.	0.7	No color	Red	....	Red

RABBIT V.

1	None	....	0.7	No color	No color	Red	Red
	0.25	5 hrs.	....	No color	No color	Red	Red
2	0.25	6 hrs.	0.7	No color	No color	No color	Pink
	0.25	15 hrs.	0.6	No color	No color	No color	Pink
3	0.5	20 hrs.	0.6	No color	No color	No color	Pink
	0.5	24 hrs.	0.65	No color	No color	No color	Pink
4	0.5	20 hrs.	0.6	No color	No color	No color	No color
	Death two hours later.						

RABBIT VI.

1	None	....	0.7	Pink	Pink	....	Red
	0.25	6 hrs.	....	No color	Pink	....	Red
	0.25	16 hrs.	0.7	No color	No color	No color	Pink
2	0.25	6 hrs.	....	No color	No color	No color	Pink
	0.25	18 hrs.	....	No color	No color	No color	No color

In this table, as in Table I, it will be seen that with increasing amounts of acid the reaction of the alkaline filtrate from the serum with phenolphthalein was frequently red in aqueous and colorless in alcoholic solution. With still further diminution in alkali the serum not only remained colorless after removal of the protein, but the aqueous dilutions of the serum did not color with phenolphthalein. This difference in aqueous

and alcoholic solution, and in the presence or absence of protein, offers the possibility of the ready detection of several different grades in the diminution of titratable alkalinity. These reactions with phenolphthalein may be listed tentatively as follows:

1. Persistence of red color in either alcoholic solution or dry residue for several hours.

2. Absence of color in alcoholic solution or transient appearance of color in the residue, but with good coloration in aqueous solution.

3. Total absence of color in alcoholic solution, in the residue on evaporation or after the addition of water, but with distinct color in the aqueous solution before removal of protein.

4. Total absence of color in all stages including the aqueous dilution without the removal of protein.

These stages would, of course, merge gradually into one another.

For convenience the sharper changes may be outlined as follows:

BEHAVIOR TOWARD PHENOLPHTHALEIN.

After removal of protein.			Without removal of protein.
In alcoholic solution.	Residue after evaporation.	In aqueous solution.	1–10 dilution of whole serum in water.
Alkaline	Alkaline	Alkaline	Alkaline
Alkaline	*	Alkaline	Alkaline
Neutral	Neutral	Alkaline	Alkaline
Neutral	Neutral	Neutral	Alkaline
Neutral	Neutral	Neutral	Neutral

* Color discharged on evaporating to dryness and heating for a few minutes.

From the results which are recorded in Table II, it is evident that a comparatively early grade of acidosis can be detected merely by the qualitative changes in the reaction of the alcoholic fraction of the serum toward phenolphthalein. These changes took place before the development of any clinical symptoms. Nevertheless, it was necessary to use rather large amounts of acid before sufficient change occurred in the blood to be detected by this method. Thus, Walther found that the introduction of 0.9 gm. of hydrochloric acid per kilo of body weight per day was fatal for rabbits. In this series it was necessary to introduce at least 0.5 gm. per kilo per day to obtain a definite effect, while in one case twice this quantity was necessary. In the advanced stages of this experimental acidosis the increasing frequency, and especially the increased depth of respiration served to compensate in part for the loss of alkali.

Walther observed that in experimental acidosis, death took place while the blood still remained slightly alkaline to litmus. The same holds true for all of the animals in this series. As regards phenolphthalein, however, the functions of the body may continue, for a limited time, although the serum after heating reacts neutral or acid to this indicator. The acid reaction to phenolphthalein of the alcoholic filtrate from the serum does not mean that the bicarbonates are absent from the serum itself, but rather that the fraction has disappeared which is removed in the alcoholic filtrate. In some instances the heated aqueous dilution of the serum remained colorless with phenolphthalein. Such sera, however, are not wholly free from alkali-yielding substances; upon incineration of these specimens in platinum crucibles at low red heat, the residue when taken up in water reacted strongly alkaline to phenolphthalein, although the aqueous dilutions upon boiling were neutral or acid in reaction. Death occurred in all cases while the serum was still strongly alkaline to phenolphthalein after incineration.

The essential feature in this series of animals consists in the demonstration of definite changes in the reaction of the blood as a result of the effect of acid in vivo, as well as in vitro. Much larger quantities, however, were required to produce a corresponding effect in the animal body than in vitro. Thus, 1 to 2 cc. of N/100 acid per 1 cc. of blood serum in vitro produced an effect corresponding to the action in vivo of the equivalent of 1400 to 3000 cc. of N/100 acid per kilo of body weight. Of course, it is not possible that the acid which was introduced was distributed equally through the various tissues of the body and it is impossible to say just what proportion acted directly on the blood itself. It is evident, however, that relatively enormous quantities must be introduced into the body to produce the same effect upon the blood as are produced by minimal quantities acting in vitro.

In considering the clinical application of this method, it is hardly necessary to note that the experimental results were obtained under conditions which differ markedly from those arising spontaneously in man. In the first place, the introduction of a mineral acid by way of the alimentary tract is rather different from the formation of organic acids in the tissues and from defective elimination of acid salts by the kidneys. Moreover, it is well known that the herbivora react in a different manner from the carnivora toward the mineral acids, while it is supposed that the omnivora constitute a somewhat intermediate group.

In order to test the applicability of this method to clinical conditions, examinations were made in normal individuals, in cases of diabetic acidosis and unknown conditions where acidosis was suspected. Of the unknown conditions, attention was directed primarily toward the study of the nephropathies. In the series of diabetics some supplementary tests were carried out in order to facilitate the interpretation of the results obtained with phenolphthalein. The complete list is as follows:

I. Examination of the urine:
   1. Determination of the ammonia coefficient.
   2. Estimation of the acetone and β-oxybutyric acid, by Shaffer's method.[6]
   3. Titration of the acidity by Folin's method.[7]

II. Examination of blood serum:
   1. Titration with N/10 acid against Töpfer's reagent.
   2. Behavior toward phenolphthalein.

[6] Shaffer: J. Biol. Chem., 1908, V, 211.

[7] Folin: Am. J. Physiol., 1903, IX, 265.

III. Determination of the effect of the ingestion or intravenous injection of sodium bicarbonate upon the reaction of the urine, *i. e.*, the tolerance to bicarbonate. The determination was carried out according to a principle which is strictly analogous to the work with sugars, *i. e.*, by estimating the amount which must be introduced into the body before it appears in the urine. Under certain conditions, when either sugars or carbonates are introduced into the body, a considerable quantity may be stored before any excretion takes place. It is recognized clearly that the cause and the manner of this storage may vary with different substances, and also for the same substances under different conditions. Throughout this paper the term "tolerance" is used in connection with bicarbonates without any reference to the explanatory factors which are involved. In the discussion of these cases it is extremely convenient to have a general term which may be used in unknown conditions without regard to the underlying explanation. For example, the expression, "tolerance to sugar," is used freely in the literature in a general sense, for it does not explain in detail the fate of sugar which is retained in the body. However, both the sugars and bicarbonates have certain important features in common. Thus, both of them may frequently be stored in the body essentially for use as such, *i. e.*, as carbohydrate and alkali. One of the striking differences between these two substances consists in the fact that relatively large amounts of sugar can be added to the diet of a normal person before any excretion takes place, whereas the addition of a little bicarbonate results in its prompt excretion in the urine. Thus, the differences between the two substances are, in part, only differences of degree. It would seem to the author to be appropriate to retain the term "tolerance" for use in a general sense with either alkalies or sugars. In case the explanation of the unknown cases is subsequently forthcoming, then it would be helpful, if possible, to select a term which would be explanatory for the case under consideration. Obviously, such a selection might prevent many difficulties. For example, in the case of the sugars, several factors may be involved in the explanation of a given case and, moreover, it is possible that the relative importance of these factors may vary from time to time in a given individual. In the case of bicarbonates, certainly no conclusive evidence has been offered which would enable one to decide upon the exact explanation of the cases. Consequently, it has seemed preferable to retain the term "tolerance" in a general sense, reserving the adoption of a specific term until the explanation of these cases is established.

Palmer and Henderson[8] have recently proposed that the excretion of bicarbonate be studied by determining the concentration of the urine in hydrogen and hydroxyl ions. By the use of a series of indicators they conclude that any individuals who require more than 4 gm. of sodium bicarbonate are abnormal. Practically identical conclusions were reached simply by the use of litmus paper.[9] The maximum amount of sodium bicarbonate was determined which is required to change the reaction of the urine in any normal adult on a mixed diet, from acid to a neutral or alkaline reaction. It was found that 5 gm. was sufficient to produce a distinct change in practically all cases. The only exceptions to this which were found were two cases of neurosis in men. On investigation of these two cases it was found that at the time when they were tested the intake of water happened to be very limited, no exercise was permitted, and the appetite was extremely poor. A second dose of 5 gm. promptly produced an alkaline urine in each patient.

In the cases reported in this paper the reaction of the urine was tested to litmus paper. In those instances in which the reaction was not distinctly acid or alkaline the specimens were boiled thoroughly in order to convert any bicarbonate into the normal carbonate. The milder cases were tested by ingestion. Ten grams of sodium bicarbonate, mixed with water, were given by mouth through a glass tube, three times daily at intervals of six hours. For the intravenous injections a 4% or 5% solution in water was injected in quantities usually of 400 to 600 cc. at intervals of about eight hours. Appropriate precautions were taken to prevent excessive change in the solutions during sterilization.[10] The bladder was emptied immediately before the ingestion or injection of bicarbonate and the reaction of the urine was tested before each repetition of the bicarbonate.

For the series of nephropathies the outline for the diabetic cases was changed slightly as follows:

I. Examination of the urine:
   1. Determination of the ammonia coefficient.
   2. Titration of the acidity of the urine by Folin's method.

II. Examination of the blood:
   1. Titration of the serum against N/10 acid with Töpfer's reagent.
   2. Behavior of the serum toward phenolphthalein.

III. Determination of the tolerance to sodium bicarbonate.

The determination of acetone and β-oxybutyric acid was omitted inasmuch as there is no reason to suspect a disturbance of carbohydrate or fat metabolism. A previous series of various types of renal disease showed no increase of these bodies in the urine.[11] Furthermore, as far as acetone is concerned under ordinary conditions, the qualitative tests when negative are sufficient to exclude any significant increase in its output.

In the series of control tests on normal individuals the only unknown factor is the determination of the behavior of normal serum toward phenolphthalein. The standard values for the other factors are so well established that they do not require repetition. The essential feature consists in the examination of the reaction of the alcoholic filtrate. In the examination of thirty normal cases it was found that the alcoholic filtrate upon concentrating gave a definite red color and, upon evaporating to dryness, this residue retained a distinct red or pink color. After several hours heating on a steam bath, the red color faded very gradually to a pale yellow. In some cases it did not dis-

[8] Palmer and Henderson: Arch. Int. Med., 1913, XII, 153.
[9] Sellards: Johns Hopkins Hosp. Bull., 1912, XXIII, 289.
[10] Sellards: *loc. cit.*
[11] Sellards: *loc. cit.*

appear till after heating for six or eight hours, and in all cases there was a distinct pink visible for at least one hour. The change in color is so gradual and so slow in normal cases that it would be impracticable to set any exact time limits for its disappearance.

It would seem evident from these data that in all normal individuals the unheated alcoholic filtrate of the serum is neutral, or slightly acid, to phenolphthalein, and becomes alkaline upon heating. The individuals on which these tests were made included all ordinary ages, except the first decade of life. However, the subsequent tests on pathologic cases did not include any children. It seems most improbable that healthy children would show any marked variation from the adult in such fundamental characteristics as the alkalinity of the blood.

The first series of pathologic conditions which were tested was a group of seven diabetic patients, in four of whom (A, B, C and D) acidosis was present, while in three others (E, F and G) it was absent. Of the acidosis cases, two (C and D) were in an advanced stage with all the typical signs. The other two were in an earlier stage without clinical signs and without any definite increase in the excretion of acetone and related bodies, or of ammonia. The diagnosis of acidosis in these two cases was based on a well-marked increase in the tolerance to sodium bicarbonate (on intravenous injection). The behavior of their titratable alkalinity is shown in Table III.

TABLE III.

CASES OF DIABETES.

	Urine.				Tolerance to sodium bicarbonate (grams).	Blood serum.				
							Behavior toward phenolphthalein.			
							After removal of protein.			1-10 dilution of whole serum in water.
Case.	Acidity in % of N/1 solution.	NH_3 coefficient.	Acetone, grams per liter.	β-Oxybutyric acid (grams per liter).		Alkalinity to Töpfer's in % of N/1 solution.	In alcoholic solution.	Residue after evaporation.	In aqueous solution.	
A	5.5	5.6	0.010	0.04	40	0.8	No color	No color	Pink	Pink
B	6.3	6.1	0.011	0.10	30	0.9	No color	No color	Red	Red
C	4.5	11.7	2.20	3.3	50+	0.7	No color	No color	Faint pink	Faint pink
D	4.0	16.4	3.20	10.5	80+	0.8	No color	No color	Faint pink	Faint pink
E	3.5	6.0	0.100	0.26	5	0.8	Red	Red	....	Red
F	4.0	5.7	0.135	0.38	10	0.9	Red	Red	....	Red
G	3.0	3.4	0.412	0.21	10	0.8	Red	Red	....	Red

In Table III the behavior of the serum toward phenolphthalein parallels the tolerance to sodium bicarbonate and bears no constant relation to any of the other factors recorded in the table. The parallelism between the behavior of the serum to phenolphthalein and the increase in the tolerance to bicarbonate in two patients (A and B) justifies the diagnosis of acidosis in these cases, if one considers acidosis to be an impoverishment of the body in bases. Moreover, the total daily output of ammonia averaged 0.4 to 0.5 gm. per day in each case and did not exceed the normal range of 0.4 to 0.6 gm. daily. We have, therefore, the existence of a definite acidosis without any appreciable increase in the relative or absolute excretion of ammonia. The behavior of the titratable alkalinity was investigated in a group of nephropathies, selecting primarily those cases which show an increase in tolerance to sodium bicarbonate. The special object in view was to determine if the bicarbonate in these cases is retained on account of an acidosis or if it accumulates in the body on account of the inability of the kidney to excrete it.

There are a number of features which make it probable that this increased tolerance is to be explained by a deficit of bicarbonate in the body. Nevertheless, it is equally clear that no crucial evidence from any standpoint has been obtained for the determination of this question. One suggestive point is

TABLE IV.

CASES OF CHRONIC RENAL DISEASE.

IN UREMIA.

	Urine.		Tolerance to sodium bicarbonate (grams).	Blood serum.				
					Behavior toward phenolphthalein.			
					After removal of protein.			1-10 dilution of whole serum in water.
Case.	Acidity in % of N/1 solution.	NH_3 coefficient.		Alkalinity to Töpfer's in % of N/1 solution.	In alcoholic solution.	Residue after evaporation.	In aqueous solution.	
A	3.5	3.0	160+	0.6	No color	No color	No color	No color
B	2.3	3.5	40+	0.9	No color	No color	Pink	Red
C	...	...	30+	0.6	No color	No color	No color	Pink
D	1.5	2.4	70	0.8	No color	No color	No color	No color
E	3.8	5.2	30	0.8	Pink	[1]	Red	Red
F	2.7	4.0	30	0.7	No color	No color	Pink	Pink
G	2.5	3.5	40	0.9	Pink	[1]	Red	Red
EXTREME CASES.								
H	3.0	3.7	70 [2]	0.6	No color	No color	Pink	Red
I	3.1	4.2	80 [2]	0.8	No color	No color	No color	Red
J	2.5	2.6	70 [2]	1.0	No color	No color	No color	Faint pink
K	2.0	8.7	80	0.8	No color	No color	Red	Red
L	2.0	11.6	40	0.7	No color	No color	Red	Red
OUTSPOKEN CASES.								
M	4.0	7.1	30 [2]	0.9	Red	[1]	Red	Red
N	1.5	11.8	30 [2]	1.0	Red	[1]	Red	Red
O	2.7	3.0	20 [2]	1.0	Red	[1]	Red	Red
P	2.4	2.2	10 [2]	0.8	Red	Red	....	Red
Q	1.8	4.0	10 [2]	1.3	Red	Red	....	Red

[1] Colorless after five minutes. [2] By ingestion.

that the urine of a nephritic which has been rendered alkaline by large doses of bicarbonate is, after the return of the acid reaction, rendered alkaline again by much smaller doses of bicarbonate than were necessary originally. It has been suggested that this fact constitutes an almost crucial test, proving the existence of acidosis.[12] It is obvious, however, that these results are open to another explanation. Thus, in the case of substances which are known to be retained in the body on account of renal lesions, it is characteristic that they are not

[12] Palmer and Henderson: *loc. cit.*

retained quantitatively when large amounts are introduced, but after a certain maximum has been reached excretion commences in the case of practically all substances. Consequently, when an acid reaction of the urine returns after the alkalinity following a massive injection of bicarbonate, it is obvious that the effect of an additional small dose of bicarbonate cannot form a crucial test for distinguishing between acidosis and retention. Table IV shows the behavior of 17 cases of renal disease, of the chronic diffuse type tested during a stage of good cardiac compensation.

In analyzing Table IV, the most significant feature is the general parallelism between the reaction of the blood to phenolphthalein and the tolerance to sodium bicarbonate. The following outline, compiled from Table IV, illustrates this feature:

BEHAVIOR OF SERUM TOWARD PHENOLPHTHALEIN.

After removal of protein.				1-10 dilution of whole serum in water.	Range of tolerance to sodium bicarbonate (grams).
No. of cases tested.	In alcoholic solution.	Residue after evaporation.	In aqueous solution.		
2	Alkaline	Alkaline	Alkaline	Alkaline	10—
5	Alkaline	*	Alkaline	Alkaline	20 to 40
3	Neutral	Neutral	Alkaline	Alkaline	30 to 70
2	Neutral	Neutral	Neutral	Alkaline	70 to 80
2	Neutral	Neutral	Neutral	Neutral	70 to 160+

*Red color discharged in a few minutes by heat on water bath.

In this outline two cases (B and C, Table IV) are omitted, since the bicarbonate was discontinued in those patients presumably long before their tolerance was reached. Only the nephritic cases are included, as it is not unlikely that the relation between the reaction of the blood and the tolerance to sodium bicarbonate may vary according to the etiology of the acidosis. This possibility is suggested by the two fatal cases of diabetic acidosis (Table III, C and D), which did not show extreme changes in the blood, although the tests were made during the day on which these patients died. Case K, Table IV, is omitted because of the hepatic syphilis, since certain features of hepatic disease suggest the possibility of an acidosis. In this patient the tolerance is slightly higher than one might expect from the blood changes.

The gradations of changes in the blood as given in this outline can only be classified in a very general way. It is quite possible that, with accurate quantitative measurement of these changes, their parallelism to the alkali tolerance might be still more definite. This relationship between the titratable alkalinity and the increased tolerance to bases indicates that the latter is due, in part at least, to a deficit of the body in bases. It is rather more difficult to decide whether this deficit is the sole factor involved in the production of this increase in tolerance. It is quite conceivable that in addition to this deficit there may be an inability of the injured kidney to excrete bicarbonate. This question can be approached by studying the changes that take place in the blood upon the introduction of bicarbonate and their relation to the changes that take place in the reaction of the urine. Thus, when the titratable alkalinity of the blood has been restored to normal, one would expect that the further introduction of bicarbonate would change the urine from an acid to an alkaline reaction unless there is suppression of the bicarbonate by the diseased kidney.

Some of the cases in Table IV, showing a high tolerance to sodium bicarbonate were studied to determine whether the changes in the blood and in the urine took place simultaneously. The results are given in Table V. In the cases in which the bicarbonate was administered by mouth, it was given in 10 gm. quantities three times per day. The reaction of each specimen of urine to litmus was tested. The blood specimens were taken each morning before the first dose of bicarbonate. In cases where intravenous administration was employed, the blood sample was taken before each injection.

TABLE V.

EFFECT OF THE ADMINISTRATION OF BICARBONATE UPON THE TITRATABLE ALKALINITY OF THE BLOOD.

Case.	Sodium bicarbonate.	Interval since last administration of bicarbonate.	Behavior of serum toward phenolphthalein. After removal of protein. In alcoholic solution.	Residue after evaporation.	In aqueous solution.	1-10 dilution of whole serum in water.	Reaction of urine to litmus.
ADMINISTRATION BY MOUTH.							
H	.....	....	No color	No color	Pink	Red	Acid
	30 gm.	15 hrs.	Trace color	No color	Red	Red	Acid
	30 gm.	16 hrs.	Red	Red	Red	Red	Acid
	10 gm.	4 hrs.	....	....	....	....	Alkaline
I	.....	....	No color	No color	No color	Red	Acid
	30 gm.	15 hrs.	No color	No color	Red	Red	Acid
	30 gm.	16 hrs.	Red	(1)	Red	Red	Acid
	20 gm.	3 hrs.	....	....	....	....	Alkaline
J	.....	....	No color	No color	No color	Pink	Acid
	30 gm.	14 hrs.	No color	No color	Pink	Pink	Acid
	30 gm.	15 hrs.	Red	Red	Red	Red	Acid
	10 gm.	4 hrs.	....	....	....	....	Alkaline
INTRAVENOUS INJECTION.							
A	.....	....	No color	No color	No color	No color	Acid
	70 gm.	15 hrs.	No color	No color	No color	Pink	Acid
	60 gm.	10 hrs.	No color	No color	Red	Red	Acid
	30 gm.	8 hrs.	Pink	(2)	Red	Red	Acid
D	.....	....	No color	No color	No color	No color	Acid
	25 gm.	7 min.	No color	No color	Pink	Red	....
	.....	22 hrs.	Red	(2)	Red	Red	Acid
	25 gm.	16 hrs.	Red	(1)	Red	Red	Acid
	20 gm.	4 hrs.	Red	Red	Red	Red	Amphoteric
Normal	.....	....	Red	Red	Red	Red	Acid
	5 gm.	3 hrs.	....	....	....	....	Alkaline

[1] Colorless in 15 minutes. [2] Colorless in 5 minutes.

From this table it is seen that the alkali deficit is the important factor in explaining the increased tolerance to bases. It is equally clear, however, that a comparatively small quantity, perhaps 5 or 10 gm., for example, might be held back by

reason of renal retention. Indeed, it would seem very striking if, in cases where there is partial suppression of the ordinary substances, such as dyes, salts, sugars, and water, that the bicarbonate alone should escape partial suppression. A quantity such as 5 gm., however, would be without any clinical significance in comparison with the total amounts which were used.

Some evidence has been collected in regard to the rapidity with which the increase in tolerance to bases returns after the administration of bicarbonate. The period required for the return of the increase in tolerance is so long that only a few cases were available for investigation.

TABLE VI.

DURATION OF THE EFFECT OF THE ADMINISTRATION OF BICARBONATE.

Case.	First test.	Interval between tests.	Second test.			Tolerance to bicarbonate (grams).
			Behavior of blood serum to phenolphthalein.			
			After removal of protein.			
	Tolerance to bicarbonate (grams).		In alcoholic solution.	Residue after evaporation.	In aqueous solution.	
H.	70	2 weeks	Red	Red	Red	10
I.....	80	2 weeks	No color	No color	Red	40
J.....	70	2 weeks	Pink	(1)	Red	20
N	30	4 days	Red	Red	Red	10—
O	20	3 days	Red	Red	Red	10—

[1] Colorless in five minutes.

These data indicate that the effect of the bicarbonate lasts for a relatively long period as compared with diabetic acidosis. Moreover, in the cases in uræmia in which massive injections were used, there was no evidence of rapid neutralization of the injected bicarbonate. These considerations are of special interest from the standpoint of therapy.

Conditions Other than Nephropathies.—Some additional examinations were made in conditions which are of interest in connection with nephritis and uræmia. One of the more important features is the study of the various combinations of cardiac and renal disease. Especial attention has been given to two groups of cases, namely, (1) in which an acute cardiac decompensation has brought about a temporary impairment of renal function; and (2) that group in which renal lesions have brought about a more or less permanent impairment of the cardiac compensation. The various tests for renal function have been employed by different observers to differentiate these two groups; it would seem that those substances would be particularly unsuitable whose excretion is markedly interfered with by an impaired circulation. On the other hand, the more favorable tests would be those which depend upon a long-standing kidney lesion. In this connection it is interesting to note that the excretion of phenolsulphonephthalein is definitely reduced during a break in cardiac compensation, whereas the rest nitrogen of the blood rises but very little, even during long cardiac insufficiency. The changes in the titratable alkalinity in the chronic nephropathies require a long period for their development and, *a priori*, one would not expect such changes to occur in cases of pure broken compensation. Four patients were tested who were crucial cases for determining the effect of a cardiac break upon the titratable alkalinity of the blood. All of the four cases were in the stage of acute decompensation. This was accompanied by marked cyanosis. Two of these cases subsequently recovered sufficiently to return to their homes. The other two were tested when in complete coma, in extremis, during the last day of life. In these two cases the diagnosis was confirmed at section. In all four of these cases the behavior of the blood serum to phenolphthalein was distinctly normal. It is apparent that this procedure offers a certain amount of information in the differentiation of cardiac and renal cases. However, the exact limits of its value could only be determined by the examination of a large series representing a variety of cases.

Of the other conditions in which there is a dyspnœic coma without diabetes, pernicious anæmia is especially interesting. Seven cases of anæmia were examined. Three of these were secondary to hæmorrhoids. The other four were pernicious in type, two being outspoken cases, while the third (A) was in comparatively good condition. The general results were as follows:

TABLE VII.

CASES OF ANÆMIA.

Case.	Red cells per cmm.	Hæmoglobin % normal.	Tolerance to sodium bicarbonate, (grams)	Behavior of serum toward phenolphthalein.			1-10 dilution of whole serum in water.
				After removal of protein.			
				In alcoholic solution.	Residue after evaporation.	In aqueous solution.	
PERNICIOUS ANÆMIA.							
A	1,800,000	47	10—	Red	Red	Red	Red
B	1,900,000	39	70	No color	No color	Trace color	Red
C	900,000	20	40	No color	No color	Red	Red
D	1,600,000	35	60	No color	No color	Red	Red
SECONDARY ANÆMIA.							
E	2,300,000	20	30	No color	No color	Red	Red
F	3,000,000	40	10—	Red	Red	Red	Red
G	2,100,000	35	30	Red	(1)	Red	Red

[1] Colorless after five minutes.

These cases of anæmia show that, in addition to the impoverishment in cellular elements and of hæmoglobin, the titratable alkalinity is often reduced. Furthermore, in these seven cases the change in titratable alkalinity bore more relation to the grade of anæmia than to the type as determined by the morphological and etiological factors. All of these cases, except the milder pernicious one (A), showed a trace of albumin and a few casts in the urine. It would seem clear, however, that the changes in titratable alkalinity were due to the anæmia, since an even more definite grade of nephropathy gives rise to no changes in the titratable alkalinity of the blood.

On the other hand, the anæmia of the nephritic cases was altogether insufficient to account for the change in titratable

alkalinity. In some of the cases, with the highest tolerance to bicarbonate and with extreme change in titratable alkalinity, the red cell count and hæmoglobin were not reduced more than a fourth of the normal. The results were so entirely independent of the red cell count and hæmoglobin content that it was not considered necessary to record these in the table of nephritic cases.

A few miscellaneous cases were observed in which the titratable alkalinity proved to be of interest. On one occasion a man, 55 years old, was admitted to the hospital in coma with a history of anuria for 15 hours and a record of chronic nephropathy extending over 35 years. The respirations were deep and regular, but were not increased in frequency, and expiration was not prolonged. The mucous membranes were normal in color. Ten cubic centimeters of urine were obtained by catheter. An abundance of albumin and a variety of casts were present. No additional information could be obtained on further examination. The diagnosis lay between uræmia and cerebral hæmorrhage. An examination of the blood serum showed a normal condition as regards rest nitrogen and titratable alkalinity. The secretion of urine commenced in a few hours, the albumin and casts disappearing almost completely within a few days. Later a history was obtained of partial paralysis of short duration in the left arm, coming on about two weeks before admission to the hospital. The subsequent record of this case confirmed the diagnosis of cerebral hæmorrhage.

In a second instance, the patient was a man of 45, who had some knowledge of medicine. A definite nephritis of moderate grade was known to have existed for several years. This patient suddenly developed attacks of dyspnœa characterized by comparatively rapid respiration, without cyanosis, and with subjective symptoms of air hunger. The absence of any physical changes in the chest, the irregularity of these attacks and the absence of any relation to physical exertion, indicated that they were neurotic in origin. Nevertheless, the possibility of an air hunger due to renal lesions could not be excluded clinically without further observation. The examination of the blood serum at this time showed only a slight decrease in titratable alkalinity. The residue after evaporation of the alcoholic extract did not lose its red color until after heating for 15 minutes on a steam bath. This would indicate a diminution in titratable alkalinity which would be far too slight to give rise to subjective symptoms.

Brandenburg [12] divides the alkaline content of the blood into two fractions. The first is the diffusible or mineral alkali. This is small in amount and consists largely of the carbonates of the blood. The second and larger fraction consists in that which is bound to the protein. Brandenburg considers that the second fraction is subject to variation and that the diffusible carbonates are relatively constant. The behavior of the alcoholic filtrates from serum point toward the reverse conclusion, for it is largely the carbonates which are removed by the alcohol. The discrepancy, however, may be only apparent, for the statements of Brandenburg may apply more particularly to the very early changes in the titratable alkalinity of the blood.

Quantitative Determination of Changes in Titratable Alkalinity.—Throughout this paper only the more important qualitative changes have been studied. The principal objection to quantitative work on titratable alkalinity lies in the inaccuracy of all procedures due, in part, to the small amount of the substances to be measured, and also to the extremely poor end-points obtained with the indicators which are alkaline to normal serum. However, the removal of protein obviates one of the difficulties with the end-point afforded by the indicator. It would seem that the sharp reaction obtained with phenolphthalein after removal of the protein should be susceptible of expression in some quantitative form even though minute quantities of alkali are handled. Several methods suggest themselves for clinical purposes, such as direct titration, colorimetric measurement, determination of the dilution at which the color disappears in the heated aqueous dilution, or the determination of the minimal amount of normal serum which will suffice to give a color reaction in the alcoholic filtrate.

A number of titrations were carried out according to the following technique: 1 cc. of serum was diluted to 25 cc. with absolute alcohol and the mixture filtered after thorough shaking. Twenty cubic centimeters of the filtrate was evaporated to dryness on a water bath and taken up in 10 cc. of water. Five cubic centimeters of N/125 sulphuric acid were added to the residue in order to titrate subsequently for the appearance rather than the disappearance of color. The titration was carried out at boiling temperature with N/125 sodium hydroxide and phenolphthalein. The number of cubic centimeters of N/125 sulphuric acid which were neutralized by the alcoholic residue, represent the titratable alkalinity of the original serum expressed in per cent of a normal solution, or rather that fraction of the titratable alkalinity which is removed in the alcoholic filtrate. Normal serum, under these conditions, gave values varying from 0.8 to 1%, and the pathologic sera, which gave a qualitative reaction for alkali, gave results varying from normal to a trace. However, the work of keeping the dilute standard solutions accurately balanced and the comparatively slight amount of information afforded did not seem commensurate with the time involved. The test simply in its qualitative form is sufficiently delicate to detect readily a grade of acidosis which has any clinical significance. For clinical purposes, however, it would seem quite feasible to adapt some simple colorimetric standard of measurement, or perhaps reduce the amount of serum employed to the minimum which will give a satisfactory color reaction in known dilution in alcohol. Another modification which readily suggests itself is the use of the whole blood without waiting for the separation of the serum. In a few preliminary tests an alcoholic filtrate did not give an alkaline reaction with phenolphthalein when whole blood was used instead of serum alone. This might have been due partly to the hæmoglobin and also, perhaps, to the large masses of precipitate formed by the whole blood which increased the difficulty of extraction.

[12] Emerson: Clinical Diagnosis, 4th ed., Phila., 1913, p. 569.

Physico-chemical Reaction of the Blood.—The work throughout this paper has been confined to the titratable alkalinity of the blood. The study of the physico-chemical reaction of the blood in health and disease did not seem appropriate under the present conditions for two reasons: In the first place, although investigators are not yet fully agreed as to the accuracy of either the colorimetric or the electric measurements of physico-chemical reaction in the presence of protein, yet the concensus of opinion is that the reaction of the blood undergoes comparatively little change even during an acidosis. The most recent work from Sörensen's [14] laboratory with improved methods for electric measurement indicates that the compensatory processes of the body are sufficiently effective to maintain a comparatively normal physico-chemical reaction of the blood, even during an acidosis. In addition to the uncertainty which still surrounds the application of the physico-chemical methods in the study of the reaction of the blood, it is evident that the observations on the titratable alkalinity offer a much simpler procedure for clinical purposes. Moreover, the weight of evidence tends to show that it is the titratable alkalinity rather than the physico-chemical reaction which is subject to variation and which consequently is of importance from a clinical standpoint. This view was suggested by Folin [15] at an early date and at a time when the first application of physico-chemical methods to the blood were frequently interpreted as indicating that the older titration procedures were erroneous and should be abandoned.

SUMMARY.

1. Changes in the titratable alkalinity of the blood occur which give rise to distinct qualitative differences in the reaction of normal and pathologic sera to phenolphthalein. Conditions are readily obtained under which the blood serum, during an acidosis, is neutral or acid, whereas under the same conditions all normal sera are strongly alkaline.

2. The less severe grades of diminished alkalinity can be detected in a qualitative way from the behavior of sera before and after the removal of protein and by the selection of a solvent, such as alcohol, in which the ionization and hydrolysis of carbonates are diminished. The effect of protein and of the solvent upon the reaction permit a variety of combinations of these factors for detecting varying grades of diminished alkalinity.

3. Definite changes in the titratable alkalinity occur in experimental and spontaneous acidosis, in certain nephropathies, and in some anæmias. The method also affords information of value in the differentiation of certain obscure comas.

4. Cases of diabetes occur in which the excretion of ammonia and of acetone and related bodies is normal, but the titratable alkalinity is decreased and the tolerance to bases is increased. This affords proof of a definite impoverishment in bases in these cases.

5. Changes in the titratable alkalinity are accompanied by corresponding changes in the tolerance of the body to fixed bases.

6. The titratable alkalinity is of important biological significance, while the available evidence indicates that the physico-chemical reaction of the blood is maintained at a fairly constant value, even in outspoken grades of acidosis.

7. The parallelism between the diminution in titratable alkalinity and the increase in tolerance to fixed bases in diabetes and in the nephropathies affords crucial evidence that this increase in tolerance is due practically altogether to a deficit of the body in alkalies or alkali-yielding substances. The questions as to whether such a condition constitutes an acidosis will be discussed in a subsequent paper.

It is a pleasure to thank Dr. Lewellys F. Barker for an interest in this work which has been very helpful.

[14] Sörensen: Personal communication.

[15] Folin: Trans. Ass. Am. Phys., 1907, XLIX, 128.

SOME ERRORS IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS.*

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It is a curious psychological fact that the mistakes of another practitioner, when narrated with a more or less complete medical history, rarely impress the listeners in any way other than avoidable. I was well aware of this when I imprudently suggested to Dr. Hamman that I would speak to you to-night of some of my errors in diagnosis and mention a few made by other men. The only medical man who has made no mistakes is the second or third year medical student just entering upon his clinical work. Some of these mistakes of mine would never have been made had the patient and myself been able to enjoy the privilege and co-operation of such a hospital staff as you have here. The limitations of private practice must be experienced to be realized. The physician must in many cases depend entirely upon his own powers of observation for such assistance as he can command in a small town among poor patients.

A hint from a wide-awake colleague, a member of the house staff or from a keen nurse, may recall to mind another case that makes the diagnosis clear. I shall present to you a number of brief histories of patients and endeavor to help you to see how I fell into error. I am fully aware that many men, keener of observation than I, would have had more data upon which to base judgment and so probably have avoided these pitfalls.

I have no doubt that I was taught in my classes in physical diagnosis that muscle sounds could in rather thin muscular subjects simulate râles, but when I told a medical student a few months after my graduation that his disease extended over the posterior of both lungs I had forgotten this fact, and later learned that he had a just discoverable apical lesion which has remained healed for thirteen years. I had never grasped the

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fact that shoulder friction could lead me to believe that a patient had pleurisy in the interscapular region, but it did. Swallowing can produce sounds in the upper chest so closely resembling râles that I had to learn that fact also. It took me many years to learn that disease could exist for a time without physical signs and that not infrequently as the patient improved the physical signs became much more extensive.

I still make yearly some mistakes in regard to the presence or absence of a pleuritic effusion. The signs seem to me in some cases, when limited in area, to be very difficult of interpretation, and the needle is of course the only test, and that even is not infallible. More difficult still is it to determine whether or not any fluid remains in the chest after repeated aspirations. I shall narrate the history of a case a little later where I found it most difficult to decide. The heart may not be displaced and the side not increased in size.

1. Some ten or twelve years ago I had referred to me by one of the physicians in the Johns Hopkins Dispensary, a patient who had been acutely ill with what seemed like the grip. She had, as I now recall it, no temperature but some cough and expectoration and râles all over the posterior of one lung and the upper half of the opposite. She applied to me for admission to the Adirondack Cottage Sanitarium and I refused her. One month later when I re-examined her, to my astonishment I found her lungs normal. An acute process was clearing up irregularly on the two sides.

2. One hot afternoon some ten years ago I was called to a little back room to see a poor woman, thirty-five or forty years of age, with high fever, very pale and weak. She was said to have tuberculosis and I immediately suspected an acute miliary tuberculosis. The lungs showed no definite signs on repeated examinations, only a very few glands were slightly enlarged and I finally obtained her admission into the Reception Hospital, where after concurring at first in the diagnosis of pulmonary tuberculosis, Dr. Baldwin made a diagnosis of Hodgkin's disease, later confirmed by the microscopic examination of a gland excised after death.

3. About seven years ago (1907) a young man, aged twenty, was under treatment by me for pulmonary tuberculosis off and on for two years. Tubercle bacilli were present in the sputum. He then returned home and to a fast life and contracted gonorrhœa and lues. One year later he had some swollen cervical glands removed which were said to be tuberculous. A year after this, two years after I had seen him, he sent for me again, saying he had had fever of 104° F. for three days. On examination, the old scar in the neck was very evident and about it were a few small glands. The right upper and middle lobes were infiltrated and showed a slight advancement of the lesion. Otherwise the physical examination was negative except Babinsky's sign was present and the reflexes were possibly a trifle increased. Four days later the patient exhibited nervousness and twitching, more on the left side and at night (so the nurse said). There was no retraction of the head and no Kernig's sign. At this time a consultant diagnosed meningitis of which I was not sure but felt that he must have obtained data which I had overlooked. The temperature remained at 104° F. for four more days (eleven in all) and then fell by crisis to normal. The sputum was negative for tubercle bacilli; and except for an occasional trace of albumen and one finely granular cast the urine was negative. The blood contained 77% of hæmoglobin, 3600 leucocytes and 5,076,000 erythrocytes. A differential count of 250 cells showed 62.8% of polymorphonuclear neutrophiles, small mononuclears 20%, large mononuclears 13%, transitionals 3.6%. On the 7th day of the fever, a count of 200 cells showed only 39% of polymorphonuclears, 18% of large mononuclears and transitionals and 43% of small mononuclears. Three weeks later a similar blood picture was found. The complement deviation test for tuberculosis was negative once and for syphilis twice. A spinal puncture was not made. A Widal test and a blood culture were negative. After eight days of apyrexia, the temperature rose gradually to 105° F., the twitching returned and it was again thought that the patient would die, but after sixteen days of pyrexia the temperature fell once more to subnormal and the symptoms largely disappeared. After fourteen days of apyrexia a third attack with a temperature of 105° F. lasted for twenty-two days, during which he was seen by Dr. S. W. Lambert who made a diagnosis of Hodgkin's disease. The spleen at this time was enlarged, the liver dulness somewhat increased and a few glands were palpable in the axilla. He died a few weeks later.

I had sufficient data to diagnose Hodgkin's disease, and why the first consultant and I thought of and excluded nearly everything else I cannot say and offer no excuse.

4. In August, 1911, a clergyman, aged about thirty-five years, came to me saying he had begun to feel badly four months previously. One sister had had pulmonary tuberculosis and recovered. Three weeks previously he had had night-sweats, shortness of breath, fever and felt weak. After five or six weeks in the Catskills he came to Saranac Lake. He was somewhat anæmic, had lost weight and his general condition was poor. The pulmonary examination showed signs of fluid (previously diagnosed) to the level of the third rib and third vertebral spine. His sputum contained no tubercle bacilli and the urine a trace of albumen and a few hyaline casts. He had continuous fever ranging about 100° to 101° F. He was aspirated repeatedly and the fluid never showed any tubercle bacilli either by animal inoculation or by staining. However I thought the patient had a tuberculous pleurisy and treated him accordingly. Between two and three months after his arrival, a slight swelling appeared in the third right intercostal space. It grew larger and larger, redder and softer. I diagnosed a cold abscess on a rib and my assistant incised the swelling but could get no pus. A little later another appeared on the opposite side of the sternum. The glands in both axillæ and above and below the clavicle then enlarged slightly. The mental condition of the patient was very peculiar. He had constant but always changing delusions of persecution which finally largely cleared up. The mass over the sternum became larger and more irregular. A blood count showed a leucocytosis of 21,000 and in a differential count the polymorphonuclear neutrophiles were 92%, lymphocytes 7%, large mononuclears 0.5%, eosinophiles 0.5%. A bit of tissue removed before death from the mass showed the characteristic picture of Hodgkin's disease, which was confirmed by a second specimen taken after death.

I thought this patient had a tuberculous effusion and later, as I said, a cold abscess. The pathologist in this case helped make the diagnosis, but like some few pathologists he left himself a loophole large enough for escape.

When the superficial glands are enlarged the differentiation of Hodgkin's disease from tuberuculosis is at times difficult, but in two cases that I have seen the only difficulty lay in thinking of Hodgkin's disease, which by the way is not always as easy in general practice as some of the third and fourth year students imagine. One of these cases was so far advanced that she died in one week after reaching the Adirondacks. The other was operated on for so-called tuberculous glands of the neck, and the tonsil on the same side removed four years ago. The removed glands were not examined in this case or the other, which was also operated on for tuberculous adenitis, nor was Hodgkin's disease even suspected by a number of medical men who had seen the patients.

Here, then, are five cases of Hodgkin's disease, all of which

were sent to the Adirondacks for tuberculosis. In two the superficial glands and spleen were enlarged and the diagnosis should have been made and the patients kept at home. In the other three the superficial glands did not enlarge at all or only very late in the disease. An indefinite onset followed by pleuritic effusion and fever is likely to lead anyone astray. When enlarged glands occur in a tuberculous patient and do not recur after removal or remain very small, it is not easy to diagnose lymphadenoma. I have no doubt a number of cases have escaped me, but to-day I am on the outlook for such cases and that, I believe, is a large factor in their correct diagnosis.

5. In the winter of 1912, a school teacher, fifty years old, from the Middle West was sent to me suffering from pleurisy with effusion. I immediately took it for granted that a diagnosis of tuberculous pleurisy had been made, as I believe it had. He complained of great pain over the left anterior costal margin and a fast pulse. One brother died of tuberculous laryngitis. He denied lues and tripper, but had pneumonia when a child. Fourteen months before he saw me he had been treated on account of the pain for indigestion. Not improving he went to Battle Creek where fifty ounces of a clear fluid, of low specific gravity, containing no pus, were withdrawn. He returned home and tried to but could not continue work. One month previous he had nearly lost the use of his left side, and since then the left palpebral orifice has been smaller than the right. Hoarseness then occurred and loss of weight and strength. His appetite was poor and bowels very constipated. He had slight expectoration. A radiograph was said to show a thickened pleura and some intrapulmonary disease on the left with scars of an old tuberculosis on the right. He stated that he had had little or no temperature but that his pulse was fast and he was slightly short of breath and slightly aphasic. His chief complaint was pain at the left anterior costal margin, so severe that he could sleep only when he lay on his back and then only when he took analgesics. The left side was depressed, moved less, showed loss of vocal fremitus and almost flatness over most of the side. The breathing was distant, the vocal resonance decreased and there were a few scattered râles. There were possibly a few fine râles in the outer third interspace on the right. The heart was not misplaced. The sputum contained some tubercle bacilli ten days after arrival and the urine was normal. Three weeks later his voice became more husky and weak, due to a paralysis of the left recurrent laryngeal nerve. Two months later, after a dry tap in front, 400 cc. of a reddish brown fluid with many polymorphonuclear and few mononuclear cells were aspirated posteriorly. A differential blood count was normal and when five months after arrival the liver was found enlarged, a negative Wassermann was obtained. There were no glands except one the size of a hazel nut in the left axilla which decreased in size. The patient returned home and as my diagnosis of malignant tumor of the lung and pleura was not acquiesced in, a laparotomy was done and revealed a liver studded with carcinomatous nodules. He died a few days after the operation. As I said at first I thought the patient had tuberculous pleurisy but as the other signs developed the diagnosis seemed untenable. He may have had pulmonary tuberculosis also as tubercle bacilli were found in the sputum at the Adirondack Cottage Sanitarium or some mistake may have occurred as at no other time were they ever demonstrated.

This case and the case of the clergyman with Hodgkin's disease show how easy it is to be misled by the presence of fluid.

6. Two years ago a woman, aged 50, the mother of two children, was referred to me for treatment. One brother died of tumor of the lung, otherwise the family history was negative. Thirty years previously she had had for about three years, attacks of bronchitis lasting about two or three months each. Her health continued good until one and a half years ago when the left breast was removed and the axilla cleaned out for a typical mammary carcinoma. She was then well until about eight weeks previously she had a slight hæmoptysis and had ever since raised blood streaked sputum in which tubercle bacilli were found. A few physical signs were discovered at the right apex and the lymphatic glands in the right cervical region were found enlarged. Pleurisy in the right infraaxillary region developed three weeks previously and the temperature rose to 100.5° F. and 101° F. Following this the temperature varied from normal in the morning to 100° F. at night. Three weeks previously her right knee began to pain her and continued to do so. The finding of tubercle bacilli convinced the hospital authorities that she had pulmonary tuberculosis and her attending physicians were considerably chagrined as they had not discovered them on several examinations. When she came under my observation June 29, 1912, she was in bed expectorating blood and had a slight elevation of temperature. I kept her in bed and only at the end of a week did I examine her lungs, for the blood spitting continued. A few scattered rhonchi were heard over the upper lobe of the right lung. The examination of the sputum and urine were negative and I thought the patient was probably tuberculous with possibly carcinomatous or tuberculous glands in the neck on the side opposite the former tumor. Two weeks (July 12) after arrival the breathing was noted as being slightly distant and broncho-vesicular above the left clavicle but no dulness could be made out. Four days later (July 16) the breathing on the right was puerile, on the left distant over the front but of good quality. On the left back there was dulness, more pronounced below. The breathing was less marked than on the right and after coughing a few sonorous râles were heard. The heart sounds were more widely transmitted than normally. The cough had now become spasmodic and suggested intrathoracic pressure and she had difficulty in breathing. The next day there was dulness over the greater part of the left lung, with flatness below, whispering broncophony was audible to the fifth rib and eighth vertebral spine, but vocal fremitus was lessened. The breathing was distant. Three days later a dry tap was obtained. On July 28, the knee was put up in crinoline. The next day double vision occurred and several days later a marked internal strabismus of the left eye occurred. Later vomiting occurred frequently. From this time on the symptoms gradually increased until weakness lessened them. The only point of interest which developed before her death on September 3 was that she had attacks of premature cardiac contraction, apparently ventricular in origin. Her headache would increase and the pulse drop from 132 to 60 and in one-half hour rise to 120. On one occasion it dropped to 38. I diagnosed secondary carcinoma of the lungs, cervical glands, brain and knee, but the autopsy performed by Dr. Krause revealed a hypernephroma on the right side with metastases to the left lung, two or three to the right lung, to the liver, left kidney, right knee and a few nodules in the mesentery and retroperitoneal tissues. The brain and neck were not examined. A few weeks ago, the sections from the mammary carcinoma were examined by Dr. W. G. McCallum, who pronounced the tumor a typical carcinoma.

7. A youth, aged 19, from New Jersey, applied for admission to the Adirondack Cottage Sanitarium last summer. His family history and his previous history were unimportant except that he lived under poor hygienic conditions and was closely associated with a boy friend who was tuberculous. About six or seven weeks before admission (March, 1913) he began to lose appetite, weight and strength, had a slight cough and suffered from languor. One and a half months later he had night-sweats, fever and sputum. On arrival in Saranac Lake one and a half months before admission he had temperature of 101° F., night-sweats and physical signs interpreted as due to enlarged bronchial glands. After admission he grew worse and was transferred to the infirmary where his

temperature ranged from 97° to 102° F., but many days never fell to normal; his pulse varied from 100 to 120, his respirations from 16 to 28. On October 12, seven months after onset, his leucocytes were 38,000 and the polymorphonuclears 92%. A radiograph showed a shadow in the mediastinum, about five inches in breadth, some diffuse consolidation in the lower part of the right lung and some consolidation about the hilus of the left lung. The note on the right was markedly dull below the sixth vertebral spine, the breathing somewhat exaggerated over the whole front, the vocal resonance normal, a few fine râles were heard at the inner end of the fourth interspace and moderately coarse râles below the fourth vertebral spine. On the left the note was hyperresonant over the lower front, the breathing slightly distant over the front. A few scattered moderately coarse râles after coughing were heard over the front and fine râles to the third vertebral spine. The following day a fluoroscopic examination confirmed the radiograph and showed the posterior mediastinum to be free. A diagnosis of mediastinal abscess was made and the introduction of a needle was considered. The second day following there was flatness from the right of the sternum extending over most of the left front, the vocal resonance was increased and the breathing markedly weakened. The patient expectorated some dark clots of blood. The urine examination was negative and the purulent sputum never showed any tubercle bacilli. The radiograph showed that the whole mass had shifted slightly to the left. At this time a mediastinal abscess was diagnosed and the patient referred to a surgeon who diagnosed malignant growth. After several days of observation a thoracotomy revealed sarcoma of the mediastinum and left lung which was confirmed by histologic examination of a piece of excised tissue. I should have made this diagnosis but the sudden change in note led me astray as well as the sharply defined right border of the X-Ray shadow and the positive opinions of some other medical men.

In these three cases of malignant tumor of the lung and pleura, it is curious that in two tubercle bacilli were found once but never again in the sputum. One patient might have had a tuberculous focus in the lung, but in the other it was excluded at autopsy. The pleuritic effusion, the reported presence of tubercle bacilli in the sputum and the fever would easily mislead anyone in the first case, while only the presence of tubercle bacilli in the sputum in the second case led one to think of anything else than a pulmonary metastasis. In fact her attending physician suspected this, but the hospital authorities were so positive of the presence of tubercle bacilli that they yielded. When a patient comes from well recognized authorities with a diagnosis made by finding tubercle bacilli in the sputum, it is well to go very cautiously before stating that their laboratory has made a mistake. The same thing, however, probably occurred with us. When the physical signs of pulmonary tuberculosis are anomalous it is wise to check up the diagnosis made from the sputum. I ought, no doubt, to have diagnosed hypernephroma, but the patient was very fat and had I felt the tumor I would, I am sure, still have called it a metastasis, for I did not then know that hypernephromata are not rare in patients who have had carcinoma.

8. A lawyer, aged 54, complaining of pulmonary tuberculosis, consulted me some seven years ago. Several members of his immediate family had suffered from and died on account of circulatory disorders. The personal history was unimportant except that he had had a slight cough for seven years, due, he said, to tobacco. While he had been exposed, no history of lues was obtained. Three of four years before I saw him he began to lose weight and strength, but recovered and was well until several months previous, during which time he had had temperature which for a few weeks ranged from 99° to 103° F. With this acute attack his cough and expectoration had increased and his physician had found tubercle bacilli in his sputum. On examination his general condition was poor, his color not good, his radial arteries thickened, pulse 100, and his second pulmonic accentuated; temperature 97° F. The pulmonary examination showed on the right: slight dulness to the third vertebral spine with good vesicular murmur, fine râles to the clavicle and third vertebral spine after coughing: on the left the side was much contracted, the vocal fremitus increased and some dulness over the entire back; the vocal resonance was increased, broncho-vesicular breathing was present to the clavicle, coarse râles were heard in the outer fourth and fifth interspace and from the sixth vertebral spine to the base. A few fine râles were present at the base anteriorly. On these data I made a diagnosis of pulmonary tuberculosis. Three examinations of the sputum were negative for tubercle bacilli and the urine was normal. His pulmonary condition improved rapidly. His temperature was normal, pulse from 100 to 108. Two months after arrival he spat blood two or three times, had slight elevation of temperature and pain in the right lower chest. Two weeks later phlebitis occurred. One month later when his lungs were nearly normal, he had a sharp pain at the base of his neck, fell off his chair and became unconscious. On regaining consciousness he vomited such large quantities of fluid that a physician made a diagnosis of acute distension of the stomach. Later in the day a few signs of intrathoracic pressure developed and that night he died suddenly. Autopsy revealed a dissecting aneurysm of the arch which had followed the pulmonary artery well into the lungs. The lungs were otherwise normal. He had had fever, hæmoptysis, phlebitis, cough, expectoration and so-called tubercle bacilli in his sputum which his physician stoutly maintains to this day were real tubercle bacilli.

I do not see how I could have avoided this mistake.

9. Last month the name of an actress, married, aged 30, was placed upon the waiting list for the Adirondack Cottage Sanitarium by one of our examining physicians. One uncle had died from pulmonary tuberculosis. Twenty years previously she had had inflammatory rheumatism and her heart was said to have been affected. However, she did as other children and when she went on the stage as a chorus girl she danced hard and sang. Last spring (1913) she went on a tour to the Pacific coast and on the return trip became ill with cough, expectoration and fever and both in Saskatoon and later in Winnipeg was told she had pulmonary tuberculosis. After a three weeks cure in Liberty she joined a company in New York and rehearsed hard. Becoming hoarse she saw a throat specialist, who referred her to our examining physician, both of them concurring in the diagnosis of pulmonary tuberculosis. Early in December she reported at the Examining Office in Saranac Lake and was advised to go to bed on account of her fever. The physical signs at that time were very slight on the right side. She sent for me a little later on account of the fever and rheumatism in the right knee. I heard nothing in her lungs but regret to state that as the room was very cold and both the patient and myself very uncomfortable, the examination was more cursory than usual. There was some enlargement of the heart and a loud systolic murmur, transmitted to the axilla and back, with the maximum intensity over the point of maximum impulse in the fifth interspace in the mid clavicular line. She had no swelling of the ankles. She had a severe cough for which I gave her a good "tuberculosis" cough mixture, but the temperature persisted until one night I was sent for as she could not "talk straight." She knew what she wanted to say but could not say it. Her right arm became paralyzed and the knee kick was absent on the right and marked on the left side. The pupils were constantly small and sluggish. The next day at 9 p. m. she gave a cry and moaned. Kernig's sign was present in the left leg for a

time. Babinsky's sign was present in both sides. The blood pressure was systolic 115 mm., diastolic 70 mm. A spinal puncture revealed a bloody fluid not under much pressure. The second day after the onset she moved her right arm but developed stertorous breathing and died that night, having been unconscious most of the day. Autopsy revealed a hæmorrhage into the left lateral ventricle and some over the convolutions about the fissure of Rolando. Her heart showed a chronic mitral lesion and a beautiful acute endocarditis. In the right lower lobe was a firm, dry patch about 5 x 5 cm. and on section like hepatization, but containing air, while the rest of the lung was œdematous. There was no tubercle. This patch could have given some signs and misled, I believe, several physicians.

What had happened was, I suppose, as follows: An embolus had lodged in a small branch of the middle cerebral artery leading to a silent area of the brain. This area had softened and with absorption of the embolus or after the formation of an aneurysm, rupture had slowly occurred and the blood oozed out. I do not see why an apical infarct might not lead to mistakes in diagnosis. I heard of a patient who was admitted to a large New York hospital, as she had heart disease and the pulmonary tuberculosis was too advanced in the apex or apices for treatment in the Adirondack Cottage Sanitarium. As her heart condition improved her lungs finally cleared up completely.

10. In the summer of 1911, a woman, married, aged 29, from Pennsylvania, sent for me and complained chiefly of pain in the back. The family history was negative and the previous history unimportant. She lived under good hygienic conditions and had never been about cattle or stables. The present illness began about ten months previously with an indefinite attack which was attributed to a floating kidney. Two and a half months later (March, 1910) she went to Augusta, Ga., for six weeks. After her return she began to cough, but only slightly at first. The following fall (November, 1910), she was so weak she went to bed for a five months rest cure. This followed by a six weeks visit to Cambridge Springs resulted in a gain in strength and weight, but with the hot weather of June the cough increased and she felt worse. In July, eighteen months after the onset, she noticed a painful and tender swelling over the right lower thorax posteriorly. Disease of the spine or rib was diagnosed, but no fluid was obtained on puncture. In August when I saw her she complained chiefly of pain in her back. She was very thin, weak, nervous and with marked pallor. The heart was apparently normal and not displaced. The right side was much contracted, moved less, with much increased vocal fremitus to the third rib. There was some dulness over the back, most pronounced below the seventh vertebral spine and bronchial breathing to the clavicle and third vertebral spine and opposite the fifth and sixth vertebral spines with increased vocal resonance. Moderately coarse râles were heard over the side on coughing. On the left the vocal resonance was somewhat increased in the axilla. There was marked rigidity of the spine, tenderness over the ninth and tenth dorsal vertebræ and brawny swelling along the spine from the eighth to the twelfth dorsal vertebræ. I informed the husband that she had extensive disease of the lungs, probably tuberculous, and also in all probability disease of the ribs or vertebræ. The temperature ranged from 99° to 100.5° F. and the swelling slowly increased in size and softened in the center. About this time marked rigidity developed in the right abdominal muscles. The urine was normal. The leucocyte count at this time was 15,000, the differential about normal. I had a surgeon see her and he though she had empyæma, probably tuberculous in origin, and advised aspiration, saying there was nothing wrong in the abdomen. A few drops of bloody, glairy fluid were obtained on aspiration which on examination contained no tubercle bacilli, but some Gram positive cocci and rods with some Gram negative ovals. About this time the patient acknowledged that she had a little sputum which was negative for tubercle bacilli. In September, two months after her arrival in Saranac Lake, the swelling was incised and under the skin a curious, rather friable, stringy, bloody tissue was found which bled freely. The abscess contained bloody, glairy, rather thick, tenacious pus, full of minute, sulphur yellow particles, slightly less than one millimeter in diameter. They proved to be actinomycotic granules, and the organism was cultivated later and found in the sputum. She died May 12, 1912. I should have suspected actinomycosis sooner but this was only the second case which I have ever seen. The first was a patient in the Johns Hopkins Dispensary.

11. In the summer of 1912 (August 24), a married woman, aged 41, wife of a professional man, came to my office saying her lungs were diseased. The family and previous history were unimportant. The present illness dated from November, 1911, nine months previously when she had a "cold" and was in bed with cough and expectoration for two weeks. She said she had had night-sweats, fever, some hoarseness, pain on the front of the chest, shortness of breath, loss of weight and strength, and cough and expectoration. She had spat blood-streaked sputum, but thought it might be due to whooping cough, which she might have contracted from her adopted son. The temperature was 99.6° F., pulse 128, respirations 20, her general condition was poor and she had slight pallor. She said no tubercle bacilli had been found in her sputum. The pulmonary examination showed rhonchi to the third rib on each side and to the fifth vertebral spine on the right, as well as fine râles to third rib on the right. I promptly made a diagnosis of pulmonary tuberculosis and put the patient to bed. During the next month she remained in bed, had a temperature ranging from normal to 101° F., pulse 88 to 116, usually 100, and respirations 20 to 26. Her cough was severe and the sputum at times was slightly blood tinged but contained no tubercle bacilli. The lungs cleared up and only an occasional fleeting rhonchus was heard. She was very erratic. Her brother came to see her five weeks after her arrival and told me of a marital infection with lues. Two Wassermann's were positive and a couple of intravenous injections of salvarsan brought the temperature to normal and the patient returned home and has been well since.

12. Several years ago a married woman, 27 years old, the mother of four healthy children, consulted me, complaining that she had high fever (103-105° F.) much of the time for three years. Her family and previous history were negative. Three years previously, while nursing a child with typhoid fever, she began to have fever and was treated for typhoid at first and later for malaria. As the fever persisted she was sent to Asheville and then kept in bed for months. The temperature finally reached 99° F., but on return home it again rose and her physician sent her to me. She had lost strength and had night-sweats, but her weight was normal, her bowels regular. The temperature in the office was 100.8° F., pulse 116 and respirations 24. The general condition was good and there was only slight anæmia. The heart was not enlarged, the abdomen negative, the urine normal and there was no sputum. The lungs were apparently normal and a gynecological examination was negative (W. Griffith). Both she and her husband denied all symptoms of lues. I referred her to Dr. Futcher who told me the Wassermann reaction was positive, but the temperature did not become absolutely normal for a time. She, however, made a complete recovery.

13. About ten years ago, a farmer, married, aged 47, from New York State, consulted me as he had had repeated hæmoptyses. One aunt died from tuberculosis. He had had cough and expectoration for thirty years, dry pleurisy as a boy and pneumonia many times. His present illness he dated two years previously, when he had his first hæmoptysis. He had had slight hoarseness, much

cough and expectoration, some shortness of breath and some loss of strength. He was slightly pale, had a temperature of 99.3° F., pulse 96 and had lost nine pounds in weight. The physical examination revealed only a few moderately coarse râles below the seventh vertebral spine on the right. The urine showed few hyaline and granular casts and the sputum was repeatedly and always negative for tubercle bacilli. Pneumococci and influenza bacillus were looked for but the results were inconclusive. The patient finally decided to remain permanently in Saranac Lake. I saw him off and on for blood spitting and he was fairly well, still with cough and expectoration, until the fall of 1910 when he had cholecystitis and during convalescence hæmorrhage into his internal capsule on the right, which caused in a few days his death. The autopsy showed besides these complications, a chronic bronchiectasis of the right lung.

This case shows the fallacy of always accepting hæmoptysis as due to pulmonary tuberculosis, but only his history at first could have excluded pulmonary tuberculosis and that was only negative evidence.

14. In the summer of 1910, a broker from New York, aged 50, consulted me as he had had fever and felt badly. His family and personal history were unimportant. Three months previous to his arrival in Saranac Lake he had a rather atypical attack which resembled pneumonia and had not been well since. Malaise had been pronounced and cough, expectoration, fever, night-sweats, shortness of breath and pain in his right side were marked. Once or twice he felt as if his right lung was choked up. He went from place to place and early in August came to see me. His temperature was 100.8° F., pulse 120, general condition poor. On examination, dulness was marked over his upper lobe with bronchial breathing and vocal resonance markedly increased. Fine râles were heard over the back and a few over the lower back on the left. The relative heart dulness apparently extended to the right of the sternum. There was pain radiating to the right shoulder when pressure was made on the abdomen. The sputum was purulent, at times tinged with blood but always negative for tubercle bacilli. The urine was normal. I felt uncertain about the diagnosis and had a colleague see him with me. He made a diagnosis of pulmonary tuberculosis. During the month he was under my observation the physical signs remained normal on the left side, but on the right, the dulness increased slightly, the breathing over the lower two-thirds of the chest was less pronounced and moderately coarse and fine râles were present over the side. Pneumococci were found in the sputum and I wanted to give him a vaccine which he refused to take. He then went to Montreal where a little later a radiograph showed a cavity. The physical signs increased and hyperesthesia developed below the third rib and above the third vertebral spine. Operation was considered, but not done until the pulmonary abscess jointed about the fourth rib under the scapula. A pure culture of pneumococcus was obtained. The patient sank gradually and died.

I feel I should have made the proper diagnosis in this case, but many points I have brought out were not emphasized in my mind until later. I was, however, always skeptical about the condition being tuberculous.

15. Two and a half years ago a trained nurse, aged about 25, was sent to me from New York. She complained of elevated temperature and at times slight cough. She had had temperature of 100° to 101° F. for many months and had consulted the best men in Philadelphia and New York. In the latter city she fell into the hands of a surgeon who opened the abdomen from the ensiform cartilage to the pubes but found nothing. Her radiographs were negative and she was referred to me as it was thought possible she might have a tuberculous pulmonary focus. The lungs were always normal and nothing abnormal of any moment was found anywhere. The urine, the blood (including a Wassermann) and the feces were apparently negative. The patient was kept in bed and then as it did not affect the temperature she was allowed to get up. In short, the temperature persisted for about sixteen months and finally disappeared. So the diagnosis of a localized miliary tuberculosis could not be verified. She is, so far as I know, well to-day.

A physician who lives in a health resort for pulmonary tuberculosis and limits his practice largely to that disease, does not have the same opportunity for making mistakes that a man in general practice has. On the other hand he can easily fall into the error of thinking that every patient has without question pulmonary tuberculosis. Fourteen years ago we had many patients reach us in the final stages of the disease. To-day the problem is changing and we are kept busy constantly endeavoring to prove that some of our patients have pulmonary tuberculosis. We are more likely to call other disease pulmonary tuberculosis than the opposite, and my course of action has been to accept provisionally the diagnosis of pulmonary tuberculosis and to begin at once to verify it. I have told you of a few of my mistakes in physical diagnosis; I have narrated briefly cases of Hodgkin's disease, of pulmonary tumor, of cardiac disease, of lues, and several others. As I look back over them I see that my errors have been largely of omission. I knew, but I failed to act. I am convinced the greater number of mistakes in diagnosis are due to carelessness, "lack of time," "a cold room," "during another visit"—is how we put it. It is impossible, I believe, for anyone to practise long without making some mistakes and I have a rule of practice which I often repeat to myself: Whenever for a few weeks you escape making a bad mistake, expect very shortly to be guilty of some glaring blunder.

OBSERVATIONS ON THE PROTECTIVE ENZYMES OF THE BODY (ABDERHALDEN).[1]

By ERNEST G. GREY, M. D., Boston.

(From The Laboratory of Surgical Research, Harvard Medical School.)

A study of the Abderhalden protective enzymes of the body was undertaken about a year and a half ago, with the view of ascertaining their relationship to epithelial transplants. As it was necessary to be familiar with the reactions of such ferments and numerous tissues in the body, a number of different organs were prepared, following the specifications laid down by Abderhalden. Specimens of sera were gathered also from different clinical sources. Various combinations of these were then dialysed and in each case the endeavor was made to ad-

[1] I wish to express my greatest appreciation to Prof. Cushing for encouraging this work and for the necessary means with which to carry it out. I desire also to thank Drs. Louis Weed, Paul Wegefarth and I. Chandler Walker for frequent aid in supplying me with materials.

TABLE I.

Case No.	Clinical Diagnosis.	Placenta, human.	Placenta, dog.	Placenta, cat.	Kidney, human.	Kidney, dog.	Kidney, cat.	Thyroid, normal.	Thyroid, Basedow.	Thyroid, adenoma.	Thyroid, dog.	Ovary, cystic.	Ovary, normal.	Liver, human.	Liver, dog.	Liver, cat.	Pancreas, human.	Pancreas, dog.	Adrenal, human.	Adrenal, dog.	Muscle, human.	Muscle, dog.	Myoma.	Blood, human.	Carcinoma, scirrhus.	Carcinoma, glands.	Adeno-carcinoma.	Hypophysis, beef.
17	Epilepsy	.....	....	.....	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....
4	Lacerated cervix	.....	....	.....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	0	....	....	++	....	....	....	....
14	Carcinoma, stomach	.....	....	.....	....	....	....	....	....	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	0	....	....
18	Varicose veins	.....	....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....
36	Pregnancy, 5th month	+	....	.....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....
13	Pregnancy, 5th month	+	....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	0	....	....	....	+	....
11	Pregnancy, ? month	0	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	Pregnancy, 3 weeks later	+	++	++	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
21	Cardio-renal syphilis	.....	....	.....	±	....	....	....	....	....	....	....	....	+	....	....	0	....	....	....	....	....	....	....	....	....	....	....
43	Perirectal abscess	.....	....	.....	....	....	....	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	++	....	....	....	....
20	Primary optic atrophy	.....	....	.....	....	....	....	0	0	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	0
10	Pregnancy, 6th week?	++	....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	0	....
19	Suspension uterus	.....	....	.....	....	....	....	....	....	....	....	0	....	....	....	....	0	....	....	....	0	....	....	++	....	....	....	....
18	Cardiac	0	....	.....	....	....	....	0	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
40	Ureteral caruncle	.....	....	.....	....	0	0	....	....	....	....	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....
8	Salpingitis, arthritis—gonorrheal	.....	....	.....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	0	....	....	....	....	0	....	....	....	....
41	Varicose veins	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
42	Fistula in ano	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
31	Pregnancy?	0	....	+	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....
22	Plastic operation, axilla	0	....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	+	....	....	....	....
25	Pregnancy, 3d month	+	....	++	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....
33	Sarcoma tonsil	0	....	0	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....
30	Varicose veins	0	....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....
16	Dyspituitarism	.....	....	.....	....	....	....	....	....	....	....	....	0	....	0	....	....	....	0	....	....	....	....	....	....	....	....	0
15	Abdominal adhesions	0	....	.....	....	....	....	....	....	....	....	....	....	0	....	....	0	....	....	....	0	....	....	....	....	....	....	....
51	Basedow's disease	.....	....	.....	0	....	....	....	++	+	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
53	Cardiac syphilis	.....	....	.....	0	....	....	....	....	....	....	....	....	0	....	....	0	....	....	....	0	....	....	....	....	....	....	....
27	Pregnancy, 3d month?	+++	++	+++	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....
60	Diabetes	.....	....	.....	0	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....
69	Hysteria, internal gland disturbance	.....	....	.....	0	....	....	±	....	0	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
24	Thyroid struma, Basedow?	.....	....	.....	....	....	....	0	0	±	0	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
28	Pregnancy, 2½ months?	+	....	.....	0	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	0	....	....	....	....	....	....	....
68	Varicose veins	.....	....	.....	0	....	....	....	....	....	....	....	....	....	+	+	....	....	....	....	0	....	....	....	....	....	....	....
35	Hypophysis tumor	0	....	.....	....	....	....	0	....	±	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	++
50	Myomata	0	....	.....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	0	....	....	....	....	....
34	Adeno-carcinoma cæcum	0	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	....	++	....
49	Chronic nephritis	.....	....	.....	0	....	....	0	....	....	....	....	....	0	....	....	0	....	....	....	....	....	....	....	....	....	....	....
29	Pregnancy, ? month	++	....	.....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	0
7	Cardio-renal	0	....	.....	0	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	0	....	....	....	....
70	Carcinoma, cervix	0	....	±	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	++	....

TABLE I—Continued.

Case No.	Clinical Diagnosis.	Placenta, human.	Placenta, dog.	Placenta, cat.	Kidney, human.	Kidney, dog.	Kidney, cat.	Thyroid, normal.	Thyroid, Basedow.	Thyroid, adenoma.	Thyroid, dog.	Ovary, cystic.	Ovary, normal.	Liver, human.	Liver, dog.	Liver, cat.	Pancreas, human.	Pancreas, dog.	Adrenal, human.	Adrenal, dog.	Muscle, human.	Muscle, dog.	Myoma.	Blood, human.	Carinoma, scirrhus.	Carcinoma, glands.	Adeno-carcinoma.	Hypophysis, beef.
44	Myomata	0	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	0	....	....	....	....	....
26	Pregnancy, 2d month?	++	....	++	....	....	....	....	....	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
45	Chronic appendicitis	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
46	Polyarthritis	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	+	....	....	0	....
23	Healthy	0	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	0	....	....	0	....	....	....	....
54	Abortion 8 days previously	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	0	....	....	....	....	....
62	Carcinoma cervix, diabetes	±	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	0	....	0	....
5	Hypophysis, polyglandular	....	....	....	....	....	....	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0
2	Pregnancy, 2d month?	+	....	++	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	0	....	....	....	....
3	Diabetes, coma	....	....	....	±	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	±	....	....	0	....
47	Endometritis	....	....	....	....	....	....	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0
81	Amenorrhea, hypopituitarism	....	....	....	....	....	....	0	....	0	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0
82	Carinoma, breast, metastases	....	....	....	0	....	....	....	....	....	....	....	....	0	....	....	....	....	0	....	....	....	....	....	....	....	....	+	....
48	Abdominal adhesions	....	....	....	....	....	....	0	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0
6	Pregnancy, 2d month?	+	....	+	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
38	Cirrhosis, liver	0	....	....	0	....	....	....	....	....	....	....	....	0	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....
87	Necrosis, phalanx toe	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....	....	....	....	....	0	....	0	....	....	....	....	....	....
12	Not pregnant?	0	....	0	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....
39	Carcinoma, cervix	0	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	0	0	....	....
32	Chronic appendicitis	....	....	....	....	0	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
55	Pregnancy, time?	+	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....	....	....	....

TABLE II.

Dog Serum No.	Serum alone before operation.	Serum and liver before operation.	Serum removed.	Serum alone after operation.	Serum and human placenta.	Serum and dog liver.	Serum and cat liver.	Serum and human liver.
1	..	..	15 days after transplantation	0	0	0	..	..
2	0	0	3 days after transplantation	0	0	+	+	..
3	0	0	5 days after transplantation	0	0	+	±	±
4	0	0	5 days after transplantation	0	0	+	+	±
5	0	0	3 days after transplantation	0	0	++	++	+

here closely to the Abderhalden technique. Some of the earlier work was performed in cooperation with Dr. Alfred Luger, to whom I am indebted for assistance at that time. It was during this period that varying results were obtained, and an interest and lively scepticism arose regarding the specificity of such ferments. Since then some investigators, notably Heilner and Petri [2] have published similar findings and have placed themselves on record as opposed to the specificity doctrine.

[2] Heilner u. Petri, Münch. med. Wchnschr., 1913, Vol. LX, 1530.

As the study progressed, however, it was found that the more exactly the experiments were performed and the greater the precautions taken against errors the more specific the ferments appeared.

Since the specimens of blood came largely from the variable clientele of a surgical clinic, no attempt was made to develop a series of cases concerning one or more definite pathologic or physiologic states. The table (No. 1) appended here presents concisely the results obtained in a more or less heterogeneous group of cases. A brief outline of the technique is

given also, together with certain conclusions drawn from the earlier portions of the work.

The method used was that described by Abderhalden. Tissues as fresh as possible were cut into small pieces and washed with 0.8 per cent NaCl solution. They were then boiled in distilled water containing a trace of glacial acetic acid and changed and boiled in distilled water until a negative ninhydrin reaction resulted.

Before each separate test the amount to be used was again boiled in ten times its volume of distilled water and the filtrate tested with ninhydrin. About 2 gm. of the organ and 1½ cc. of hemoglobin—free serum were used. These were incubated for 18 hours at body temperature.

The membranes were carefully standardized, using the same serum and peptone solution for each set. Occasion was taken to frequently retest such sets. Care was exercised to use only perfectly clean utensils.

Manifold errors in the first months of work demonstrated the following points: It is essential that the organs be fresh; great care is necessary in standardizing the membranes and in retesting the sets frequently; it is absolutely necessary that the organs be blood-free; paradoxical results appear to indicate some error of technique such as undoubtedly crept into the investigations of Heilner and Petri; the serum must be hemoglobin-free, fresh and sterile, and collected several hours after meals; a non-specific cleavage of the protein in question usually depends upon the presence of blood corpuscle elements; liver, for example, is very hard to prepare free from blood; controls are necessary for each serum and for each organ. Observing the above precautions such controls have always been negative.

As yet we are not familiar with the part played by acute and chronic inflammatory processes in stimulating or hindering the formation of the ferments under discussion. Thus far we do not clearly understand the part played by polyglandular and polyvisceral disturbances in the formation of antiferments.

Thirty-seven sera from a variety of patients were tried with placental tissue. Twenty-two were from women not pregnant, and of these no serum reacted positively. There were twelve sera from women clinically pregnant. The tests were all positive in these cases with two exceptions. One serum, No. 31, was from a questionable case of pregnancy. It reacted strongly with cat placenta, but not with the human material. The error possibly lay with the placental preparation, which was immedately discarded and new material prepared. Another serum, No. 11, gave a negative test at first, but three weeks later broke down the same placental preparations—human and cat. Four sera from carcinoma cases were set up with placenta. Only one, No. 35, gave a feebly positive reaction.

With kidney tissue from man, dog or cat, sixteen different sera were tried. Of these four had nephropathic histories. No. 21, a cardio-renal case, with syphilis, and No. 3, from a patient in diabetic coma, gave slightly positive reactions. Another cardio-renal, No. 7, and a case of chronic nephritis, No. 49, did not react at all. No. 53, from a cardiopathic patient, gave a negative reaction.

Sixteen sera were tested with normal human thyroid. No. 69, from a case of probable internal glandular disturbance, gave a feebly positive reaction. With Basedow tissue 6 sera were used. Four had no clinical histories of thyroid disturbances. No. 51 was from a case of outspoken Basedow disease and definitely broke down the tissue. It did not affect normal thyroid. No. 69 (see above) yielded a negative test. No. 24, from a nervous patient with a struma, reacted negatively. Ten sera were tried with prepared adenomatous thyroid tissue. Of these three gave some result. No. 24 (see above) and No. 35 (hypophysis tumor) yielded feebly positive results. No. 51 (see above) definitely broke down the adenomatous tissue.

Three sera were tried with dog thyroid. All gave negative tests.

With normal human ovary twelve sera were used. Three of the tests resulted positvely: No. 24, thyroid struma; No. 51, Basedow disease; and No. 69, probably internal glandular disturbances. Three sera were used with tissue from a cystic ovary. Only one, No. 36 (pregnancy), reacted—feebly positive.

With tissue preparations from human, dog and cat livers twenty sera were tried. No. 21 (cardio-renal with syphilis), No. 14 (carcinoma of the stomach with marked liver necroses), and No. 68 (varicose veins) yielded positive results. Four of the negative sera—Nos. 25, 26, 28 and 6—were from pregnant cases. Two sera—Nos. 14 and 82—came from patients with carcinoma. The first reacted and the second did not. The donor of serum No. 38 had cirrhosis of the liver with ascites.

Ten sera were set up with preparations of normal pancreas tissue. Two were from nephritic cases and three from diabetic patients—one in coma. In no instance was the tissue broken down.

Adrenal gland preparations were used with nine sera. The tests were all negative.

In thirteen different cases (three pregnant sera) there was no serum which acted upon muscle tissue preparations.

Six sera were used against myoma tissue. Two cases—Nos. 44 and 50—had uterine myomata. In no instance was there a positive reaction.

Ten sera were tried against preparations of human blood. The positive reacting specimens were: Nos. 46 (polyarthritis); 43 (perirectal abscess); 19 (24 hours after a laparotomy); 22 (6 days after plastic operation on axilla); 4 (24 hours after curetage and repair of cervix); and 3 (diabetic coma) feeble reaction.

With preparation of adeno-carcinoma of the cæcum ten sera were used. Of these five had clinical (and pathological) carcinoma. Three yielded positive results. Two, Nos. 39 and 62 (from carcinoma cases) gave no reaction. Six sera were tested with lymph gland metastasis preparations. All resulted negatively. Two of these, Nos. 39 and 14, were from patients with carcinoma. One serum, No. 33, was from a case of carcinoma of the tonsil. Six sera with a scirrhus carcinoma preparation (from the breast) yielded negative results, except one case, No. 34, which was feebly positive.

A beef hypophysis preparation was set up with eight sera. Of the latter, four were from dyspituitary cases. Three were negative (Nos. 5, 20 and 16). One reacted positively (No. 35).

BLOOD FERMENTS FOLLOWING TRANSPLANTATIONS.

In December, 1912, a case in the surgical service of the Johns Hopkins Hospital was operated upon—sellar decompression—for an hypophyseal cyst. The patient manifested a syndrome of hypopituitarism, with accompanying diabetes insipidus. Several months later, because of the marked pituitary insufficiency, a second operation was undertaken, consisting of a subcortical implantation of the hypophysis of a newborn child. Improvement in the glandular signs appeared for a short time. Subsequently, however, the features of the hypopituitarism became marked, and the patient finally succumbed.

The improvement noted was attributed by the operator to either the material contained in the glandular transplant or to the secretion manufactured by the transplant itself during its period of absorption. Could the graft have survived indefinitely it seemed plausible that the patient might have recovered, in part at least, from the state of hypopituitarism.

Similar negative results were obtained by Exner[3] in transplanting the hypophysis in rats.

The laws governing the viability of epithelial transplants are still very obscure, in spite of much work in this direction. The literature yields opinions, however, nearly unanimous in the view that homo- and hetero-transplants of the parathyroid gland are absorbed ultimately as foreign material.[4] Halstead's[5] dog, which maintained good health with a parathyroid autograft approximately ¼ mm. in diameter, is a striking example of its kind.

In brief we may mention the following as some of the possible factors in the failure of such transplantation work.

1. A "Verhungern" of the transplant.[6]

2. A toxic action of the transplant on the host. Such a condition is found in certain cases of blood transfusion.[6]

3. A toxic action of certain constituents of the hosts plasma upon the graft.[7]

4. The lack of a necessary degree of deficiency in the host, of the gland in question.[8]

5. A trophic sequence.

6. An immunity reaction—in the sense of an anaphylactic reaction,[9] or otherwise.

The fact that at first the transplants frequently become vascularized and only later are absorbed argues against a permanent interference with the blood supply in all cases. Carrel's[10] work on the kidney has shown that such an organ may survive transplantation in the same animal and functionate, provided the circulation be maintained. This, in part, answers the fifth point above, namely, that certain glands can probably functionate successfully without their nerves and after a temporary suppression of their special secretory duties.

The second, fourth and sixth points may be considered under the heading of a "defensive mechanism," in the sense of Murphy[11]—a mechanism whose strength and rapidity of reaction depend upon the degree of relationship, being more prompt and violent the more foreign the tissue introduced. Murphy thinks that this defensive mechanism is a property developed rather than one naturally present in the host.

Russell[12] has shown that the cells of a mouse survived and multiplied in a normal rat for more than nine days, while in a rat previously immunized the graft was rapidly disintegrated. Furthermore, Lambert and Hanes[13] have added the observation that rat and mouse tissues will grow almost as well in plasma from an alien as from the native species. If the animal, however, from which the plasma is obtained is previously immunized with the living cells of the foreign species, the plasma will inhibit or actually prevent any such activity.

In view of the protective ferment mechanism of the body demonstrated by Abderhalden in other fields, the observations above mentioned suggested a possible cytolytic action on the graft of antienzymes developed in the host following the transplantation. It appeared conceivable that the hypophyseal graft considered above caused the formation of proteolytic enzymes which, in turn, brought about its ultimate absorption. As the patient had previously received glandular therapy, both intravenously and by mouth, we are able to conceive how such glandular administration may have lessened the chances for the ultimate survival of the foreign pituitary body.

With this idea in mind an endeavor was made to use the Abderhalden technique in detecting such antiferments as might be present after the transplantation of an epithelial organ. The liver was chosen because of its size and ease of access. As the accompanying table (No. 2) shows, five dogs were used in the experiment. In each case, with one exception, the animal was bled previous to the transplantation and the serum tested for ferments capable of breaking down liver protein. It was negative in each case. In the first dog—the exception—a preliminary test was not made, but since the ultimate result proved negative, this has no significance.

In Dog I a small piece of liver, roughly 7 or 8 gm., from another dog just sacrificed, was implanted under the rectus muscle. The wound healed *per primum*. Fifteen days after the operation the blood serum was tested against dog liver. The result was negative. The length of time lapsing between the transplantation and the test and the method of grafting

[3] Exner, A.: Deutsche Ztschr. f. Chir., 1912, CVII, 172.

[4] Leischner, H., and Köhler, R.: Arch. f. klin. Chir., 1910, XCIV, 169.

[5] Halsted, W. S.: J. Exp. Med., 1912, XV, 205.

[6] Schöne-Marburg: Wien. klin. Wchnschr., 1911, XXIV, 734.

[7] Axhausen, G.: Med. Klinik., 1911, VII, 1801.

[8] Halsted, W. S.: J. Exp. Med., 1909, XI, 175.

[9] Schöne-Marburg: *loc. cit.*

[10] Carrel, Alexis: J. Exp. Med., 1910, XII, 146.

[11] Murphy, J. B.: J. Exp. Med., 1913, XVIII, 491.

[12] Russell, B. R. G.: Third Scientific Report of the Imperial Cancer Research Fund, 1908, 341.

[13] Lambert, R. A., and Hanes, F. M.: J. Exp. Med., 1911, XIV, 129, 453.

the liver tissue were held accountable for the failure in this experiment.

In Dog II about 15 gm. of liver were removed from a recently killed dog, cut into small pieces and implanted under the rectus muscle. Three days later the serum was set up with dog and cat liver tissue. They both reacted positively.

In Dog III about 15 gm. of liver were removed from a dog, cut into very small pieces and implanted under the rectus muscle of the donor. After five days the serum was tested for proteolytic ferments. It reacted with dog, human and cat livers.

In Dog IV about 15 gm. of liver were removed as in Dog III, cut into fine pieces and transplanted under the rectus muscle of the same animal. With dog, cat and human liver preparations the serum reacted after five days.

In Dog V about 10 gm. of liver tissue ground up in salt solution (from another dog) were injected intraperitoneally. Three days later enzymes were present in the dog's serum capable of breaking down dog liver.

Human placenta was used as a control in these dialysations. Such controls were always negative.

These results seem to indicate that liver tissue, under certain circumstances, when transplanted into a dog, acts as foreign protein in the animal's circulation and stimulates the formation of proteolytic enzymes capable of digesting it. Transplants of this nature, then, have to cope with protective ferments developed in the body of the host.

Between the experiment as carried out in Dogs II, III and IV, and the experiment represented by Dog V there is no essential difference other than one of quantity. Where the gland tissue has been reduced to a semi-fluid form we expect it to have a greater capacity for stimulating the formation of antienzymes than an equal mass of gland tissue sectioned into pieces. Just so a transplant of less proportions, an hypophysis for example, probably brings about a protective ferment formation of comparatively limited extent, a formation entirely commensurate with its size. There is every reason to believe then that the process is the same in each case. From these considerations we are fairly safe in concluding that a small graft as well as a larger one is capable of stimulating the formation of a protective mechanism in the body of the host.

Since the subcutaneous,[14] intraperitoneal,[15] and oral administrations[16] of protein all stimulate the formation of ferments capable of breaking down such a protein into its cleavage products, it is clear that procedures of this nature, if instituted before or during a transplantation, will serve to aggravate the antiferment formation evoked by the graft itself.

CONCLUSIONS.

1. The results of this work agree with the conclusions of those investigators who have found that the Abderhalden ferments are specific.

2. In transplanting epithelial organs it is necessary to remember that such a procedure stimulates the formation of antiferments in the host; and, in the case of glands, it is important to avoid any preliminary intravenous or subcutaneous feeding—perhaps oral also—of the substance, since such administrations encourage the development of protective enzymes on the part of the host.

[14] Frank, E., u. Rosenthal, F.: München. med. Wchnschr., 1913, LX, 1594.

[15] Fuchs, A.: München. med. Wchnschr., 1913, LX, 2230.

[16] Bauer: Wien. klin. Wchnschr., 1913, XXVI, 1109.

PRINCIPAL TYPES OF MICRO-ORGANISMS IN BALTIMORE MILK.

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During the past year an attempt has been made to describe and classify the most frequent and important organisms found in Baltimore milk. The studies on pasteurization now being made in this laboratory, and the attempt to demonstrate the ætiological relationship between certain intestinal infections, notably typhoid fever and the summer diarrhœa of children, and an impure milk supply, render such a study of great importance, as it is manifestly impossible to determine the fate of pathogenic organisms or to explain the effect of heat of various degrees on milk without some notion as to the bacteria which predominate in the milk in any given community.

The organisms which we have encountered have been described as far as possible in such a way that their identification by other workers in this field may be comparatively easy. No great care has been expended upon descriptions of colonies in agar or gelatin, or the manner of growth of the organisms in these media, on shades of pigment, or upon a number of other properties of bacterial growth which are variable, and depend on inconstant properties of the media employed, and which are frequently of so elusive a nature as to be incapable of description. On the other hand stress has been laid upon those biological activities which are usually constant, and whose presence or absence may be indicated by positive or negative characters.

The samples of milk examined were collected from small shops in the northeastern part of the city, with the exception of two specimens obtained in bottles from local dairies. Plates of agar were poured at once and the predominant colonies picked up after twenty-four to forty-eight hours incubation at 37° C. The samples of milk were also incubated for twenty-four hours, and a second set of plates made from which colonies were also picked. When for any reason there arose suspicion of a mixed culture, plating was again resorted to, or whenever the colonies were so numerous that there seemed danger of transferring a mixed culture, the colonies themselves were again plated, and well separated colonies transferred to agar.

The organisms from these agar growths were then inoculated into litmus milk, gelatin, potato and neutral broth, and into dextrose, lactose, and saccharose broth in fermentation tubes. With certain organisms which failed to grow well in broth, agar, to which the various sugars were added, was substituted for the fermentation tubes. Of the media mentioned potato was found to be of the least value from the standpoint of constancy and clearness in description, but was used throughout because of its importance in a few instances, and out of regard to former descriptions.

Only twelve different samples of milk were studied, since no attempt was made to isolate and describe all the rarer bacteria existing in milk, and it was found that the common types were encountered in the first few specimens examined. The later samples revealed the same organisms, or variants of the same differing in no very important details. About forty different strains were isolated, and we believe that these include the most common varieties of bacteria existing in Baltimore milk.

The organism most frequently encountered, and the one which seemed to exist in the greatest numbers corresponded to a type described by Günther and Thierfelder,[1] Leichmann,[2] and others as a bacterium, and by Grotenfeldt[3] and others as a streptococcus, and which is commonly known as *B. acidi lactici, Bact. lactis acidi,* and as *Streptococcus lacticus.*

Great confusion exists in regard to this organism on account of the fact that different investigators have given it various names, while others have given a similar name to a distinct, separate species. Thus Hueppe[4] has named *B. acidi lactici* an organism which grows like *B. coli,* produces gas in dextrose, forms spores, and peptonizes milk, and Grotenfeldt[5] and Zopf[6] independently have described bacteria indistinguishable from *Bact. ærogenes* and *B. coli* which they have called *B. acidi lactici,* and Perkins[7] has taken this type, and made it a definite subdivision of the Friedländer group. At the present time the term *B. acidi lactici* has very little meaning. If used at all it should apply undoubtedly to the organism described by Hueppe, which grows luxuriantly on agar, and forms gas in dextrose, and which according to some authors is in reality *Bact. ærogenes* (Escherich) Migula, and which according to Perkins differs from the latter only by its inability to ferment saccharose.

The other type which is the predominant one in the Baltimore milk, also sometimes known as *B. acidi lactici,* grows very faintly on agar, produces no gas in dextrose, and according to some authors is a bacterium, and in the opinion of others is a streptococcus. Günther and Thierfelder [l.c.] first described it, but erroneously identified the organism with the one previously described by Hueppe. Leichmann [l.c.] first recognized the difference between the two, but very unfortunately named the organism *Bact. lactis acidi* in contradistinction to the *B. acidi lactici* of Hueppe. The similarity of these two names, and the fact that Günther and Thierfelder had identified the bacterium, later called *Bact. lactis acidi* by Leichmann, as the *B. acidi lactici* of Hueppe resulted in endless confusion, many authors continuing to apply the latter term to the organism which should bear the former appellation.

To make matters worse, other observers described apparently the same bacterium, but called it by various names. This is well shown by the fact that Matzuschita[8] regards the terms *Bacterium güntheri, Bacillus lactis acidi* Liebmann, *Bacillus der spontanen Milkgerinnung* Günther and Thierfelder, as synonyms for *Bacillus lacticus* (apparently the *Bacillus lacticus* of Kruse[9]), and states that the *Bacillus leichmanni* (*Bacterium lactis acidi, Milchsaurebacillus* of Leichmann) is probably identical with it.

In addition, the curious morphology of the *Bact. lactis acidi* of Leichmann made the situation even more involved. For it is evident from the literature that there exist either two organisms of identical cultural characters, one a bacterium, the other a streptococcus, or else that the morphological appearance of a single organism has been differently interpreted by various observers. Thus Leichmann [l.c.] named the bacterium of Günther and Thierfelder *B. lactis acidi,* and Liebmann,[10] Kozai,[11] Utz,[11] Esten,[11] and Schierbeck [11] seem also to have described the same organism as a bacterium. But Kruse [12] called attention to the resemblance which it bore to the pneumococcus, and suggested that a better term would be *Streptococcus lacticus,* though he acknowledged that rod-shaped forms existed.

Then Grotenfeldt,[l.c.] Weigmann [13] and Freudenreich [14] described streptococci which seem identical with this *Streptococcus lacticus* of Kruse, and finally Marpmann [15] named apparently the same organism *Sphærococcus acidi lactici.*

The name *Streptococcus lacticus* given by Kruse has been generally adopted, and has found its way into various textbooks, but has the serious disadvantage of ignoring the bacterial form, and of placing the organism definitely in the class of streptococci, thus raising the question of its relationship to the *Streptococcus pyogenes.* On this account and in order to avoid the use of the term *Bact. lactis acidi,* which has been the cause of so much confusion, the writer in this paper has substituted the name *Bact. güntheri,* used first by Lehmann and Neumann in May, 1896, to designate the organism described by Günther and Thierfelder,[l.c.] but incorrectly identified by them as the *B. acidi lactici* of Hueppe. According to the rules of nomenclature this term should take precedence over the name *Bact. lactis acidi* later given to the same organism by Leichmann, and to the names *Bacillus lacticus* (Flügge [l.c.]) and *Streptococcus lacticus,* also later applied by Kruse.

Some strain of this bacterium was isolated from every specimen of milk examined. Usually it was found as the predominant type in curdled milk, but not uncommonly it seemed to be the most numerous variety in fresh milk as well.

In regard to morphology and cultural reactions, the writer's results differed in no important detail from the description given by Kruse. The organism when examined in a hanging drop made from a 24-hour dry agar slant culture presented an appearance which varied in different cultures. Some strains were composed of slender, short rods. In others the shape and size were so dissimilar that the appearance was that of a mixed culture, as has been noted by Bartlett.[16] For some of the forms were broad, some slender; some had pointed ends, others had ends rounded; some were so short as to closely resemble cocci; others were two or three times as long as broad. Single forms,

pairs, short chains and clumps all added to the picture of a contaminated culture. But repeated platings failed to change this appearance, so that it is evident that but one organism was present.

On the other hand other strains when examined in the same way seemed at first glance to be genuine diplococci or streptococci, and at first the writer thought it possible to classify the various cultures into two distinct types, one a polymorphous bacterium, the other a streptococcus. But careful search always revealed streptococcus forms in the bacterial type, and bacterial forms in the streptococcus type, while so many strains were intermediate in character that finally the writer was forced to the conclusion that no definite line could be drawn between the two varieties.

When examined in smears stained with gentian violet the likeness to streptococci became even more marked. The rod-shaped forms under these conditions appeared mostly as diplococci, and as noted by Kruse resembled closely the pneumococcus. In milk the various strains also appeared as diplococci and as short streptococci, and in plain broth and in the sugar broths long chains were frequent.

All of the strains isolated agreed in growing faintly on meat extract agar, in producing no hæmolysis on rabbit blood agar, in being Gram positive, in showing no motility, nor capsules, nor spores, and in being facultative anærobic, growing even better in the stab than on the surface of agar. In addition, they grew at a temperature of 20° C. as well, though more slowly than in the thermostat at 37° C. This important fact was early recognized by Kruse l.c. in his discussion of the relationship of this organism to the pneumococcus.

The colonies in agar appeared at the end of twenty-four to forty-eight hours in the thermostat as minute pin points and pin heads, which, under the microscope, appeared homogeneous and translucent. Some strains grew in broth in the form of a flocculent precipitate without turbidity, while others produced a faint cloudiness in this medium. On potato there could be observed at times a barely perceptible growth; at other times no visible reaction could be perceived. None of the strains liquefied gelatin, though they all grew well along the line of the stab. In regard to milk, the action was variable, as has also been noted by Kruse.l.c. Sometimes this medium was coagulated with a strongly acid reaction and reduction of the litmus after twenty-four hours in the thermostat. At others only a slight, though permanent, acidity was produced, even at the end of two weeks or more. At first this was thought to indicate definite strains, but it was found that the power of coagulating milk was not a constant quantity with this bacterium. When a culture which possessed this quality was plated, it was observed to give off strains which failed to coagulate the milk, though otherwise identical with the original organism, and one culture which at first failed to coagulate, after several month's growth on agar assumed this power.

The action of the organism upon dextrose, lactose, and saccharose broth was found to vary with its power of growth in these media. But when agar was used as a base the result became constant for each particular strain, and consisted in all cases in the production of acidity in dextrose and lactose. In saccharose some strains caused acidity, while others did not. No gas was ever observed.

When injected subcutaneously into rabbits and guinea pigs in large doses no apparent harm resulted. A number of guinea pigs and rabbits were thus treated with broth emulsions made by scraping and washing the growths of freshly isolated strains from twenty-four hour slant agar cultures. At the end of four weeks these animals seemed as healthy as ever.

The viability of the organism was tested by transplanting every month from agar slant to agar slant for one year. Under these conditions the organism continued to live, and four strains so kept gave at the end of this period the same reactions as at the beginning. A fifth strain which at first failed to coagulate milk later was able to do so.

The thermal death point of these strains in milk was roughly estimated after nearly a year had passed from the time of their isolation, and strangely enough was not found to be uniform. Thus four of the cultures were killed when transferred to milk and immediately heated in a water bath kept at 60° C. for fifteen minutes. A fifth strain when repeatedly treated under identical conditions survived a temperature of 65° C. for fifteen minutes, but was killed by 70° C. for the same length of time. Morphologically, this latter culture belonged to the streptococcus type of *Bact. güntheri* and seemed to be otherwise identical with types whose thermal death point was under 60° C. The fact that one strain showed in milk a thermal death point of between 65° C. and 70° C. would indicate that some strains of this organism are capable of surviving pasteurization.

On account of the importance attached by many departments of health to the presence of streptococci, and especially of the *Streptococcus pyogenes* in milk, and because of the resemblance which this organism bears to *Bact. güntheri*, it may be well to consider here the question of the identity of the two species. Kruse l.c. first called attention to the great similarity existing between the lactic acid bacterium of Leichmann and the *Streptococcus lanceolatus*, and proposed for the former the term *Streptococcus lacticus*. Heinemann [11] in a recent paper states that "the *Streptococcus lacticus* agrees in morphological, cultural, and coagulative properties with pathogenic, fecal and sewerage streptococci," and further concludes that "since *Streptococcus lacticus* is invariably present in market milk, and in fresh milk collected with good precautions, the sanitary significance of streptococci in market milk will need further investigation."

With this latter statement we agree thoroughly, but in our opinion there do exist definite morphological and cultural differences between *Bact. güntheri* (called *S. lacticus* by Kruse and Heinemann), and the *Streptococcus pyogenes*, which is evidently the organism meant under the term "pathogenic Streptococci." This point is important from a public health standpoint. For if *S. pyogenes* agrees in its morphological and cultural properties with *Bact. güntheri*, it is evident that the identification of *S. pyogenes* becomes impossible. And since *Bact. güntheri* is practically always present in milk, even

of high quality, it follows that the isolation of an organism having its cultural and morphological properties ceases to have any sanitary significance.

But according to our observations *Bact. güntheri* and *S. pyogenes* show the following differential points:

First. Morphologically *Bact. güntheri* usually presents some rod-shaped forms when examined in a hanging drop made from an agar slant, and some strains show very little resemblance to streptococci under these circumstances. *S. pyogenes* on the other hand is commonly a definite streptococcus under such conditions.

Second. *Bact. güntheri* never hemolyzes blood agar in our experience. *S. pyogenes* usually does so.

Third. *Bact. güntheri* lives for at least a month on meat extract agar kept at room temperature. *S. pyogenes* on this medium under these conditions dies in about ten to fourteen days, or even in a shorter period of time, and indeed may not grow at all.

Fourth. *Bact. güntheri* grows on favorable media at a temperature of 20° C. *S. pyogenes* requires a higher temperature.

Fifth. One of the strains of *Bact. güntheri* isolated by the writer possesses a thermal death point of between 65° C. and 70° C. *S. pyogenes* has a thermal death point of 54° C. according to Sternberg [18] and Hartmann.[19] At times it may be possible to differentiate the two organisms by this means alone.

Sixth. Some observers state that *Bact. güntheri* is not pathogenic for rabbits and guinea pigs, although others differ in regard to this. Heinemann [19] admits "that streptococci freshly isolated from milk, apart from those originating from mastitis, do not possess high virulence," but claims that this property may be acquired. In the writer's experience *Bact. güntheri* is non-pathogenic for rabbits and guinea pigs even when inoculated in huge doses from freshly isolated strains. *S. pyogenes* when freshly isolated usually is pathogenic for these animals.

In addition, it should be remembered that much of the confusion existing in regard to the various properties of *S. pyogenes* may arise from the fact that many of the atypical cultures recovered from tonsils, the mucous membranes of the mouth, and other sources touched by milk, with which *Bact. güntheri* is readily confused, very possibly may be in reality *Bact. güntheri*, and not the genuine *S. pyogenes*.

If these observations are correct it follows that *S. pyogenes* may be identified and separated from the organism which seems universally present in milk, and which it so closely resembles. In consequence the isolation of a culture having the typical morphology and cultural reactions of *S. pyogenes* should have some significance from a public health standpoint. But it must be admitted that *S. pyogenes* and *Bact. güntheri* resemble each other so closely in their cultural reactions and morphological features that even after isolation in a pure culture they can be distinguished only after careful study. In addition, the two organisms considered are practically indistinguishable in milk by morphological means, though sometimes presenting minor differences, such as length of chain formation. In consequence, any diagnosis of *S. pyogenes* in milk made solely on the basis of microscopic examination must be very dubious in character.

Next may be described a species which in many ways was very similar to *Bact. güntheri*, and for this reason is considered here. This organism, which probably may be identified best with the one mentioned by Conn [20] as *B. lactici ærobans*, was found three times in fresh milk, and on two occasions seemed the predominant type present. It was a bacillus which resembled the bacterial type of *Bact. güntheri*, and like the latter sometimes presented the appearance of a mixed culture. At times when examined in a hanging drop made from an agar slant preparation many of the forms seemed to be short, fat rods with rounded ends. Others were so short as to appear circular, and yet others were beyond doubt rather thin bacilli. Short chains were common. Like *Bact. güntheri* the organism was Gram positive, and possessed neither capsules, nor the power of spore formation. At first it appeared to be non-motile, but after several months growth on agar there was observed a definite rotatory motility quite distinct from Brownian motion, which placed it definitely in the class of the bacilli. Culturally the organism differed from *Bact. güntheri* by its action on litmus milk, in which it produced no apparent change, and upon lactose agar in which it caused no acidity. Otherwise it was identical with the saccharose fermenting type of *Bact. güntheri*.

After *Bact. güntheri*, which is of constant occurrence in Baltimore milk, the most frequently encountered single organism was *Bacterium ærogenes* (Escherich) Migula l.c. * (*B. lactis ærogenes*). It was present in half the samples of milk examined, twice in fresh, and four times in incubated milk. It is significant that half the specimens did not reveal the organism on the plates, and it is reasonable to conclude that it was either lacking from the samples, or, what is more probable, present in comparatively small numbers, since the colonies of this bacterium are large and well developed on agar plates, and not readily overlooked. The organism had classical morphological and cultural reactions, showing itself under the microscope when stained from a milk preparation as a large encapsulated bacterium, positive to Gram. It acidified and coagulated milk without peptonization, failed to liquefy gelatin, produced gas on potato, and fermented all three of the carbohydrates employed. This organism is now usually classed in the Friedländer group. It is worthy of note that the corresponding encapsulated bacterium, *Bact. acidi lactici* as described by Perkins, l.c. was not encountered, all the other fermenting bacteria which were found being probably strains of *B. coli*.

In addition to *Bact. ærogenes* various bacteria connected with the intestinal tract were isolated with a fair degree of regularity. The non-saccharose fermenter *B. coli* (Escherich) Migula was found in four samples of sour milk, and the saccharose fermenter usually now referred to as *B. coli communior*, according to Durham's suggestion, was found three times in fresh and twice in sour milk. Several of the strains of *B. coli*

* P. 396.

when first isolated were quite devoid of motility, even in 18-hour cultures in broth, and such organisms naturally resembled the *Bacterium acidi lactici* (Perkins) of the Friedländer group. But with the exception of one strain all such cultures developed definite motility after cultivation in the laboratory for a time, and were thus clearly to be regarded as *B. coli*, and the one strain in which no motility was ever demonstrated failed to show any mucoid growth on agar, and at no time could we determine in it the presence of capsules. This culture we came to regard as representing the *B. coli immobilis* of Germano and Maurea,[n] since it has all the cultural reactions of *B. coli communis* of Escherich, but is devoid of motility. Then an organism which was identified as *B. zenkeri* (Hauser) Migula l.c. * (B. proteus zenkeri) was obtained three times, once from fresh and twice from sour milk. This was characterized by branching colonies on agar, and a branching penetrating growth on an agar slant and in gelatin. It was positive to Gram, was motile, and showed no spores. On potato, it produced a dirty brown growth; gelatin it failed to liquefy, and milk it rendered alkaline, without coagulation or peptonization. In dextrose, lactose, and saccharose broth it produced neither acidity nor gas. No characteristic cultures of *B. proteus vulgaris*, nor of *B. proteus mirabilis* were ever isolated.

In addition to these well-marked types, a number of non-pigmented, non-spore bearing bacteria were isolated whose characteristics we usually associate with the organisms of the intestinal tract, and for this reason are described in this paper, although they cannot be said to occur frequently in the Baltimore milk. These bacteria present great difficulty in proper classification. They have doubtless been described by other observers as occurring in milk, but if so the descriptions are too inadequate or indefinite to be made use of. They correspond closely to species described by Ford [m] as existing in the intestinal tract, and while the absolute identity of the two sets of organisms, one derived from the intestinal contents, the other from milk, cannot be proven, we prefer to follow the descriptions given by this author. These organisms were as follows: *Bacterium oxygenes*, isolated in one sample of sour milk; *Bacterium cæci*, isolated from one sample of fresh milk, and *Bacillus chylogenes*, which was found as the predominant organism in one sample of milk, both when fresh and sour.

Bact. oxygenes was Gram negative, showed no motility or capsules, and formed no spores. It had the appearance of *B. coli* on agar, produced a luxuriant growth on potato, and made broth turbid. It failed to liquefy gelatin, and coagulated litmus milk with the production of whey and of a pink color that seemed to indicate acidity. Yet neither in lactose nor saccharose broth did it form any acidity or gas. In dextrose broth it produced acidity without gas, and in this differed from the *Bact. oxygenes* of Ford, which made this medium alkaline.

B. cæci like the preceding was Gram negative, formed no spores nor capsules, and showed no motility. But on agar it appeared translucent and moist, and on potato and in broth it failed to grow visibly. It liquefied gelatin slowly, and peptonized milk with an alkaline reaction, and without coagulation. In dextrose, lactose, and saccharose agar it produced neither acidity nor gas. Except in regard to motility this organism corresponded very closely to the *B. cæci* of Ford.

B. chylogenes was of especial interest, since it appeared in large numbers in the one specimen of milk in which it was found, and indeed seemed to be the predominant organism. It was Gram negative, possessed no capsule, formed no spores, and was violently motile. All of its reactions were very slow in forming, appearing only at the end of from three to ten days. On agar the bacillus at first grew faintly, but later resembled *B. coli* in its general appearance. It flourished luxuriantly on potato, and produced turbidity in broth. It liquefied gelatin, and coagulated milk. It produced neither acidity nor gas in lactose and saccharose. In dextrose it formed acidity without gas. It will be seen that this organism corresponds to the *B. chylogenes* of Ford, except for its growth on potato.

Associated with these organisms may be placed a species which occurred in half the samples of milk examined, five times in fresh specimens and once in an incubated specimen. Although it was never the predominant organism, it was a clear-cut variety, with definite cultural reactions, and was comparatively easy to recognize. In its cultural reactions it closely resembled the *B. fæcalis alkaligenes* of Petruschky, from which it differs in regard to motility, morphology, and Gram reaction. It also resembled the organism described as *B. No. 66* by Conn l.c.* and the *B. Troilii* (*Bact. lactis longi* b) mentioned in Matzuschita, l.c.† with which perhaps it may best be identified.

Examined in the hanging drop this organism appeared as a rather fat bacterium, about twice as long as broad, with rounded ends and without motility. No capsule was demonstrated. When stained by the Gram method it retained the gentian violet. On agar its growth resembled closely that of *B. coli*. Its colonies in this medium after twenty-four hours incubation appeared as minute pin points and pin heads, opaque when deep, delicate and translucent on the surface. However, in forty-eight hours these grew to be quite large, and were often indistinguishable from those of *B. coli*. In gelatin and on potato it likewise resembled *B. coli*, causing no liquefaction of the former medium, and forming a moist, heavy, brownish growth on the latter. But in milk and in the sugars employed its action was quite different. In these media it produced no acidity. No gas was formed in dextrose, lactose or saccharose, and litmus milk inoculated with this organism gradually became more and more alkaline, but was never peptonized, or coagulated. In meat extract broth it usually failed to grow, although one strain succeeded in producing a slight turbidity in this medium.

A variety of this bacterium was found on three occasions in fresh milk. It differed from the more common type in producing acid in dextrose, and in causing no visible growth on potato.

The most difficult organisms to classify satisfactorily, and a

* P. 816.

* P. 54.

† P. 376.

group encountered with great frequency, being in fact next to *B. leichmanni* in this respect, were the various species of micrococci. By far the larger number resembled closely the pyogenic staphylococci, from which often they could be differentiated only by their manner of growth on agar, a characteristic very difficult to describe clearly. The various strains belonging to this type differed but little in cultural reactions, and could be included in one group whose various members are apparently identical with the organism described by Zimmerman [2] from water (*Micrococcus cremoides*), and by Dyar [3] from air (*Micrococcus aureus*). For convenience of description, they will be placed in one group, which will be named after the coccus first isolated by Zimmerman (*M. cremoides*).

One or more members belonging to this general type were isolated from ten specimens of fresh milk out of the twelve examined, but were never the predominant organism, though occasionally occurring in large numbers. The different strains agreed in being Gram positive, and often showed a variation in size and in intensity of color when stained with the ordinary analine dyes. It was not unusual to see in a smear from a fresh, carefully plated culture forms so markedly different in size and in depth of staining that it was impossible to believe in the purity of the preparation. Yet invariably subsequent plating failed to change this appearance. At the present time, after several months growth on agar, this peculiarity has disappeared, and only rather large staphylocci are found in the cultures.

The various strains appeared on agar plates as large, coarse, opaque, white or yellowish colonies. On agar slants they grew luxuriantly, at first white, but later often turning to a dirty yellowish tinge, the agar itself assuming a brownish color. In gelatin liquefaction along the line of the stab was produced in from forty-eight hours to ten days, while on potato there usually appeared in twenty-four hours a flat, white or yellowish growth, at times faint, at others profuse, probably dependent upon some unknown factor in the ingredients of the potato.

In milk the different members of the group varied somewhat, for while the majority acidified and coagulated the medium with the expression of so much whey that peptonization was suggested, and may indeed have been present, yet others produced only a soft acid coagulum, with little or no whey.

All of the types formed acidity without gas production in dextrose and lactose agar, but in saccharose, while some produced acidity in twenty-four hours, others failed to do so after seven days incubation.

The only other coccus found more than once during the course of the examination showed tetrad forms, and could be classed with the sarcinæ. It closely resembled the preceding group in its manner of growth on agar and in its general cultural reactions, but differed somewhat in its action on milk and gelatin. The former medium it acidified, but failed to coagulate, while gelatin it liquefied in the form of a cup, rather than along the line of inoculation, as was the case with *M. cremoides*. It was recovered three times from fresh milk, and so possibly may be classed among the more common organisms, but could not be identified with any accuracy with any sarcina forms previously described and named by other observers. For this reason and on account of its close resemblance to the *M. cremoides* type, the writer has preferred to place it as a subvariety of this group.

The organisms just described probably include the most common species existing in the Baltimore milk, capable of growing ærobically at body temperature on ordinary media. But in addition, there were encountered a number of classified and unclassified cocci, bacteria, spore bearers, and yeasts similar to those found in the air and water. Their occurrence was inconstant, and they were present usually in small numbers. Each specimen of milk examined revealed one or more new varieties, but it was felt that their rarity and scarcity made their detailed description a matter of little value. However, it is worthy of note that on two occasions yeasts seemed to be the predominant type in curdled milk, being apparently more numerous than *Bact. güntheri*, and that one of these forms, though the predominant type in curdled milk, yet failed after isolation to produce any but an alkaline reaction upon this medium.

This yeast grew rather faintly upon agar, produced no pigment, and was Gram positive. It failed to liquefy gelatin, and made milk alkaline in much the same way as *B. alkaligenes*. In dextrose and saccharose broth it produced acidity and gas, which consisted almost entirely of carbon dioxide. In lactose broth neither acidity nor gas was observed.

The other variety of yeast occurring in large numbers in one sample of curdled milk was identical with the preceding, except for its action upon lactose and milk. The latter it acidified and coagulated, while in lactose broth it produced both acidity and gas.

CONCLUSIONS.

1. The most frequently encountered organism in the milk of Baltimore belongs to the type described by some as a bacterium, by others as a streptococcus, and which is variously known as *B. acidi lactici, Bact. lactis acidi, Bact. lacticus,* and as *S. lacticus.* In the writer's opinion, *Bact. güntheri,* a term first used by Lehmann and Neumann, is the correct name.

2. Certain strains of *Bact. güntheri* closely resemble *S. pyogenes,* but in our opinion may always be differentiated from this coccus.

3. Certain strains of *Bact. güntheri* possess in milk a thermal death point of between 65° and 70° C. and so are theoretically capable of surviving pasteurization.

4. *Bact. ærogenes* (Escherich) Migula (*B. lactis ærogenes*), and the various strains of *B. coli,* next to *Bact. güntheri,* were the organisms most frequently met with. They are more easily recovered from curdled milk, but are also frequently isolated from fresh samples.

5. No *B. acidi lactici* of the Friedländer type was encoun-

tered during the examination, and it seems possible that this organism described as occurring frequently in milk may correspond to the *B. coli immobilis of* Germano and Maurea.

6. Other organisms resembling *B. coli* in many ways may be isolated from time to time, and sometimes are the predominant type in the sample of milk collected. Their identification presents great difficulties. For the sake of clearness they have been regarded and described as identical with some of the rare organisms found in the intestines.

7. A bacterium resembling *B. alkaligenes* in its cultural reactions, but perhaps best spoken of as *Bact. Troilii,* is quite common in fresh milk in this vicinity.

8. The micrococci in milk are found in such great variety that any satisfactory classification is difficult to make. The majority may be grouped together as the cremoides type first described by Zimmerman. They are organisms apparently derived from air and water, and usually are recovered from fresh milk.

9. The remaining organisms isolated, while presenting interesting features in special cases, are too inconstant and usually too few in number to make their description a feature in a paper of this character.

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BACTERIOLOGICAL FINDINGS IN BALTIMORE OYSTERS.

By Morris Joseph, B. S.

(From the Laboratory of Hygiene and Bacteriology, The Johns Hopkins University.)

While but a few cases of typhoid fever have been traced with clearness to the use of oysters contaminated with sewage—such cases occurring, as a rule, in epidemic form as reported by Conn[1]—the opinion has been gaining ground that much of our winter typhoid fever has its origin in sewage-polluted bivalves. This opinion is strengthened by the careful study of the occurrence and distribution of the cases of typhoid fever in such a city as Washington. The recent observations of Gorham,[2] however, throw some doubt upon the wisdom of attributing winter typhoid to this source, for he has shown that during cold weather oysters go into a condition of rest or hibernation, when the ciliary movement ceases and feeding does not occur, and the oysters become practically free from sewage organisms, even when lying in sewage-polluted beds. A bacteriological study of the oysters sold in Baltimore was undertaken, therefore, to determine whether they contain organisms derived from the intestinal tract and whether their content in bacteria varies with the season of the year.

Twelve different lots of oysters, with five in each lot, were examined. They were purchased in various shucking houses, where they were said to come from four different areas—Chesapeake and Lynnhaven bays and the York and Rappahannock rivers. This examination indicates the quality of oysters at the time they are sold, and not at the time of collection. The methods employed were those adopted by the Committee on Standard Methods of Shell Fish Examinations of the American Public Health Association. When the oysters were collected at the shucking houses, certain oysters with deep bowls were purchased and carried to the laboratory, where five of the most suitable were selected for examination. The outside of the shell was first thoroughly washed with running water to free it from dirt and then allowed to dry in the air. It was then sterilized by exposure to the free flame, especially along the thin edge, after which the oyster was opened with a shucking knife, also sterilized in the free flame. The mother liquor or shell juice was immediately drawn off in sterile pipettes and dilutions of 1-10, 1-100 and 1-1000 made up with sterile water. From these dilutions dextrose fermentation tubes were planted to determine the presence of fermenting bacteria, intestinal in origin, and agar plates poured to determine the approximate number of bacteria in each oyster. Finally, those fermentation tubes, in which an active evolution of gas had taken place, were plated and characteristic colonies transferred to agar. Colonies were also transferred from the agar plates used for making the counts to agar and both sets of cultures identified by means of the ordinary culture media.

In determining the quality of the oysters examined the standard method already referred to was followed. By it the oysters are scored in the following manner: The presence of *Bacillus coli* or some other gas producer in each of the five

TABLE I.

BACTERIOLOGICAL SCORING OF BALTIMORE OYSTERS.*

Source and date.	Oyster.	Dilution 1-10.	Dilution 1-100.	Numerical value.	Agar count per cc.
Rappahannock River, Lot I, 10-29-'12.	1	0	0	0	2,833
	2	0	0	0	2,193
	3	0	0	0	547
	4	+	+	100	2,533
	5	0	9	0	1,033
				Score 100	
Chesapeake Bay, Lot II, 11-2-'12.	1	+	+	100	63,866
	2	+	+	100	3,900
	3	+	+	100	75,666
	4	+	+	100	57,666
	5	+	+	100	23,333
				Score 500	
York River, Lot III, 12-3-'12.	1	+	0	10	6,700
	2	+	+	100	20,133
	3	+	+	100	9,560
	4	+	+	100	24,466
	5	+	+	100	2,560
				Score 410	
Rappahannock, Lot IV, 1-14-13.	1	+	0	10	No count.
	2	+	0	10	Do.
	3	0	0	0	Do.
	4	0	0	0	Do.
	5	0	0	0	Do.
				Score 20	
York, Lot V, 1-20-'13.	1	0	0	0	12,000
	2	+	0	10	80,000
	3	0	0	0	12,000
	4	0	0	0	15,000
	5	0	0	0	39,000
				Score 10	
Rappahannock, Lot VI, 1-28-'13.	1	0	0	0	No count.
	2	0	0	0	Do.
	3	0	0	0	Do.
	4	+	0	10	Do.
	5	0	0	0	Do.
				Score 10	
Chesapeake, Lot VII, 2-3-'13.	1	0	0	0	2,000
	2	0	0	0	0
	3	+	0	10	No count.
	4	0	0	0	1,000
	5	0	0	0	1,000
				Score 10	
Lynnhaven, Lot VIII, 2-6-'13.	1	+	+	100	No count.
	2	+	0	0	16,000
	3	+	0	0	25,000
	4	+	+	100	1,132,000
	5	0	0	0	0
				Score 200	
Chesapeake, Lot IX, 2-11-'13.	1	+	0	10	No count.
	2	0	0	0	12,000
	3	0	0	0	No count.
	4	0	0	0	3,000
	5	0	0	0	0
				Score 10	

*The names of the various lots of oysters employed in this table must not be taken too literally, since there is no proof that the oysters were actually collected in the various areas according to which they are labeled. Indeed there are some grounds for believing that the names are merely trade-names employed to indicate the size of the oysters and have no relation to their source of collection.

TABLE I—Continued.

BACTERIOLOGICAL SCORING OF BALTIMORE OYSTERS.*

Source and date.	Oyster.	Dilution 1-10.	Dilution 1-100.	Numerical value.	Agar count per cc.
Lynnhaven, Lot X, 2-18-'13.	1	+	0	10	3,000
	2	0	0	0	No count.
	3	+	0	10	1,000
	4	+	+	100	3,000
	5	+	+	100	1,000
				Score 220	
Rappahannock, Lot XI, 4-1-'13.	1	+	+	100	14,000
	2	+	0	10	13,000
	3	0	0	0	1,000
	4	+	+	100	12,000
	5	+	+	100	11,000
				Score 310	
Rappahannock, Lot XII, 4-16-'13.	1	+	+	100	13,000
	2	+	+	100	51,000
	3	+	+	100	16,000
	4	+	+	100	16,000
	5	+	+	100	8,000
				Score 500	

*The names of the various lots of oysters employed in this table must not be taken too literally, since there is no proof that the oysters were actually collected in the various areas according to which they are labeled. Indeed there are some grounds for believing that the names are merely trade-names employed to indicate the size of the oysters and have no relation to their source of collection.

oysters in any lot is given a value which represents the reciprocal of the greatest dilution in which the gas test is positive. For example, if gas is present in a dilution of 1-10, the numerical value for the oyster is 10; if present in a dilution of 1-100 it is 100. The numerical values of the five oysters in each lot are added, in order to give a score to that oyster-bed for that particular time of the year. The tests upon which the score is based are only presumptive ones, but in most cases are confirmed by the cultural reactions of the organisms subsequently isolated. The various lots of oysters were scored according to this method and the scores are given in Table I. The agar counts for each oyster are also included.

A number of suggestive points are brought out in considering the results tabulated above. It may be noted first that the bacterial findings indicate that the oysters sold in Baltimore are in general free from sewage contamination. Thus six out of the twelve lots may be regarded as entirely satisfactory, having scores of 100 or less and showing less than three positive fermentation tests out of five in 0.1 cc. portions. Such lots of oysters would be regarded as free from pollution according to the standards of the Bureau of Chemistry of the United States Department of Agriculture and by the Rhode Island Shell Fish Commission, which bodies have adopted the most rigid regulations in America.[3] Of the lots which would be condemned by these standards, two, Nos. XI and XII, from the Rappahannock River, were collected in April, the last month of the oyster season, at a time when the weather was quite warm. Of the four other lots whose score was such as to indicate sewage contamination, one, No. II, was collected early in November. This leaves only three lots of oysters in the entire number examined in cold weather which would be

condemned by the most rigid standard. When it is remembered that some time necessarily elapses between the time of collection and time of sale, during which period the microorganisms in the oyster have an opportunity to multiply, these findings must be regarded as pretty favorable.

The most striking point brought out in this table is the distinct change in the character of the oysters, according to the time of the year. Thus in the early fall the scores were high, in the cold weather of midwinter low, and again high in the spring. This is indicated by striking an average for the various lots of oysters as is done in the following table:

TABLE II.

SEASONAL VARIATION OF BACTERIAL FINDINGS.

Source.	Average Fall Score.	Average Winter Score.	Average Spring Score.
Rappahannock	100	15	405
York	245	10	No exam.
Chesapeake	500	10	No exam.

Here it may be seen that during the cold winter months the scores of the three lots of oysters examined at different seasons are all low and the oysters free from contamination. These results confirm in a general way the opinion of Gorham and raise a serious question whether the typhoid fever which occurs in Baltimore during the cold months of winter can reasonably be attributed to oysters. Finally, it may be noted that there is a distinct correlation between the number of bacteria in each oyster and the number of gas producers as determined by the fermentation tube. A low bacterial count is associated with absence of fermentation, and a high count with a fermentation produced by considerable fractions of a cubic centimeter. This seems to indicate that the increase of bacteria which the various oysters show is in large part due to a multiplication of the intestinal organisms and not to an increase of the ordinary water species. This correlation between bacterial count and fermentation is indicated in Table III. It should be noted that no such correlation exists ordinarily in milk, as we have shown in a previous paper.[4]

VARIETIES OF MICROORGANISMS FOUND IN BALTIMORE.

Many of the oysters examined revealed the ordinary spore-bearing and pigmented bacteria which are characteristic of water and have no significance in this connection. In addition a number of non-spore-forming, non-pigmented species were isolated from time to time. These organisms were worked over with care and their cultural peculiarities established on the usual media. In some instances their identification presented no difficulties. Thus *Bacillus coli* was obtained 14 times in 17 oysters with positive fermentation tests. It had the classical reactions of motility and decolorization by Gram's stain, acidifying and coagulating milk, failing to liquefy gelatin, producing indol, and fermenting the carbohydrates with the evolution of a gas consisting of a mixture of hydrogen and carbon dioxide, with an excess of the former. Organisms differing from *Bacillus coli* by their gas formula, producing an excess of carbon dioxide over hydrogen and thus corresponding to *Bacillus cloacæ* of Jordan, were obtained five times. They differed from Jordan's *Bacillus cloacæ* in not liquefying gelatin, but should probably be included in this group.[5] Their acidification and coagulation of milk was also much slower than that of *Bacillus coli*. *Bacillus alkaligenes* of Petruschky was isolated from one oyster. This organism also presents no difficulties in its recognition, since its alkaline reaction in milk and its failure to liquefy gelatin or to act upon any of the carbohydrates place it quite definitely. In addition to these well-defined species, a number of forms were encountered which presented some difficulty in classification. The most frequent of these was an organism with some of the reactions of *Bacillus coli* and others of *Bacillus cloacæ*. Thus it splits up the carbohydrates in the same manner as *Bacillus coli*, with a gas preponderating in hydrogen over carbon dioxide, and acidifies and coagulates milk with great rapidity. It thus resembles *Bacillus coli*. At the same time it causes a rapid liquefaction of gelatin in a funnel-shaped growth along the

TABLE III.

CORRELATION BETWEEN FERMENTATION AND BACTERIAL COUNT.

Lot No.	Sample No.	Bacterial count.	Dextrose dilution 1-10.	Fermentation 1-100.
I.....	1	*2,833	0	0
	2	2,193	0	0
	3	547	0	0
	4	2,533	+	+
	5	1,033	0	0
II.....	1	63,866	+	+ (Also in 1-1000)
	2	3,900	+	+
	3	75,666	+	+
	4	57,666	+	+
	5	23,333	+	+
III.....	1	*6,700	+	0
	2	20,133	+	+
	3	9,560	+	+
	4	24,466	+	+
	5	2,560	+	+
V.....	1	12,000	0	0
	2	80,000	+	0
	3	12,000	0	0
	4	15,000	0	0
	5	39,000	0	0
VIII.....	2	16,000	+	0
	3	25,000	+	0
	4	1,132,000	+	+
	5	0	0	0
X.....	1	3,000	+	0
	3	1,000	+	0
	4	3,000	+	0
	5	1,000	0	0
XI.....	1	14,000	+	+
	2	13,000	+	0
	3	1,000	0	0
	4	12,000	+	+
	5	11,000	+	+
XII.....	1	13,000	+	+
	2	*51,000	+	0
	3	16,000	+	+
	4	16,000	+	+
	5	8,000	+	+

*Indicates where gas should have appeared in one or both dilutions, but failed. These exceptions only number 3 out of 38 samples tabulated, approximately 8%.

line of inoculation, resembling *Bacillus cloacæ*. This organism is identical with the organism isolated by Ford and Watson [6] from the Baltimore city water and described by them as a liquefying fermenter. It was isolated five times in our work and was a well-defined species. In addition, we found on various occasions bacteria which agreed in their cultural reactions with some of the rarer intestinal species originally described by Ford [7] from the intestinal tract. The most important of these were: *Bacterium oxygenes*, an organism acidifying and coagulating milk, producing no liquefaction of gelatin and growing only in the open bulb of the fermentation tube; *Bacillus oxyphilus*, acidifying and coagulating milk, not liquefying gelatin and producing acid from dextrose growing in the closed arm of the fermentation tube; two species identified as *Bacillus chymogenes* and as *Bacillus chylogenes*, acidifying and coagulating milk, liquefying gelatin and producing acid from dextrose, with growth in the closed arm in one case, but without it in the other. While these organisms were found on but a few occasions, their cultural reactions were quite distinct and the species were well marked. Whether they have any significance in oysters cannot be decided at the present time. Species like *Bacillus coli* and *Bacillus cloacæ*, however, and probably the liquefying fermenter of Ford and Watson, are surely indicative of sewage pollution of the oysters from which they are isolated. This is further borne out by the fact that these organisms were isolated from those lots of oysters which would be condemned by the methods of scoring described above. In this connection, it should be noted that on no occasion were organisms of the type of *Bacillus typhosus* or *Bacillus paratyphosus* encountered.

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BRIEF NOTE ON THE BALTIMORE CITY WATER.

By Marjorie Dietz.

(From the Laboratory of Hygiene and Bacteriology, The Johns Hopkins University.)

In a recent paper by Ford and Watson * it was pointed out that the chemical treatment of the Gunpowder water, the main source of the Baltimore City supply, had not resulted in as great a diminution of intestinal bacteria as was to be expected from the experience of other cities. The number of bacteria in the water was markedly diminished, but the fermenting organisms continued to appear with some frequency. On January 1 a different method of applying the chemical treatment was adopted, alum being added first, followed by the hypochlorite of sodium. This treatment of the water was applied at the Montebello reservoirs instead of at Loch Raven. It seemed of interest, therefore, to make a further study of the city water to determine whether this change in the method of applying the chemical treatment had resulted in any change in the character of the water. Our examinations were carried out over a period of three months and gave results which were entirely different from any heretofore obtained in this laboratory. During this time the bacterial count has been surprisingly low, averaging only fifteen colonies to the cubic centimeter. On several occasions only six or seven colonies were to be found in the plates and on no occasion has there been a count of more than twenty bacteria per cubic centimeter. The type of bacteria has completely changed, moreover, only the spore-bearing organism characteristic of the after-growths seen in chemically treated water being found. The conclusions to be drawn from the agar plates were confirmed by the fermentation tubes, which were uniformly negative for fermenting organisms in one cubic centimeter quantities and usually negative in five and ten cubic centimeters. On three occasions active fermentation occurred with ten cubic centimeters and on two occasions with five. Plates poured from these fermentation tubes revealed no ærobic colonies of the intestinal type, only the ordinary spore-bearers. The suspicion arose, therefore, that the fermentation in these tubes was not due to ordinary intestinal fermenters, but to some spore-bearing anærobe, possibly the *Bacillus ærogenes capsulatus* or "gas bacillus" of Welch and Nuttall. This suspicion was confirmed by the inoculation of a rabbit with material from one of these tubes. The rabbit was killed and its body placed in a warm room for twenty-four hours. After the lapse of this time characteristic gas œdema was present throughout and the organism was obtained in pure culture from the blood.

As a result of our examination of the city water, it can be stated that the change in the method of applying the chemical treatment has resulted in a marked change in the character of the water, the intestinal bacteria disappearing almost entirely. The city thus is in a position to purify its water supply by chemical treatment and eliminate its water-borne typhoid fever. Such a practice is to be recommended on all public health grounds, but on no account should the chemical treatment be regarded as a substitute for sand filtration. It should serve only as an emergency procedure till the system of sand filters is installed and put in complete operation.

* Ford and Watson: Johns Hopkins Hosp. Bull., 1913, XXIV, 226.

PROCEEDINGS OF SOCIETIES.

THE JOHNS HOPKINS HOSPITAL MEDICAL SOCIETY.

October 1, 1913.

Two Cases of Helminthemesis (from the Out-Patient Department of the Johns Hopkins Hospital). DR. DAVID I. MACHT, Instructor in Medicine, The Johns Hopkins University.

The following two cases which I had occasion to study at the Johns Hopkins Dispensary last summer are sufficiently exceptional to deserve reporting.

CASE No. F. 8193.—J. W., male, white, married, age 25 years, American, farmer. Was admitted to the Dispensary June 19, complaining of "indigestion, pains in the stomach, and *vomiting of worms.*"

Family History.—Negative.

Past History.—Negative. Bowels have been regular prior to the present illness.

Present Illness.—Has been suffering for the past four years from indigestion, which takes usually the form of irregular pains more or less severe, of variable duration, coming on especially after eating. His bowels are irregular, usually constipated, necessitating the use of Epsom salts. Appetite, however, has always remained good. Has lost 15 lbs. in weight, in the last three months. For the last four weeks appetite has been ravenous. "The more I eat, the more I want," as he says. In spite of the good appetite he has lost weight, been nauseated, and vomited a number of times in the latter part of the day.

Three weeks ago, after severe retching, he vomited a worm, which he described as having the appearance of a fish worm. He never noticed any worms in the stools before that time, but has since. Feels weak, and complains of pains around the waist line.

Physical Examination.—Looks thin, pale, and feverish. Tongue heavily coated; foul odor from the mouth.

Temperature 100.5° F. Pulse, 120 to the minute, regular, of moderate volume and tension.

Heart and Chest.—Negative.

Abdomen.—Flat and symmetrical. No tenderness on pressure, no masses felt. Liver and spleen not enlarged. Stomach is distended by gas and rises high in the left axilla.

Urine contained no albumin or sugar.

Diagnosis.—From the history of the case, a provisional diagnosis of ascaris infection was made.

The patient was given 1 gr. doses of santonin, and two days later brought some ten or twelve full-size specimens of ascaris lumbricoides which he had passed, thus confirming the diagnosis.

CASE No. F. 5242.—R. K., female, white, married, age 21 years, was admitted to the Dispensary on May 19, 1913. Her complaint was "tapeworms."

Family and Past History.—Negative.

Present Illness.—In December, while in the early months of pregnancy, patient was vomiting frequently. One day after a violent retching she *vomited a portion of a tapeworm.* Becoming terribly frightened she called to her mother who ran to her assistance and pulled the worm out of the patient's mouth. The segment thus expelled was about one and a half yards long. Following the shock of fright, the patient had a miscarriage. Two days later she passed two long pieces of worm per rectum, and has since then for half a year been passing small segments in the stools.

At present patient complains chiefly of weakness and nervousness. She sleeps well and has no pains. Appetite is not very good; bowels are irregular. No spasms or convulsions. No more vomiting.

Urine.—No albumin and no sugar.

Diagnosis.—From the history of the case a provisional diagnosis of tapeworm was made. This was confirmed by the therapeutic test. The patient was given two ½ drachm doses of oleoresini aspidii and two days later brought a jar full of tapeworms. On examination of the segments it was found to be a tænia saginata. The head could not be found.

The above two cases are remarkable for the vomiting or expulsion of intestinal parasites by mouth, complained of in both, which we may term "Helminthemesis." Vomiting of worms is not unknown, and yet is considered a rare occurrence. The commonest cases on record are those of ascaris lumbricoides. This *nematode* may wander into various organs of the body and cases are reported of its getting into the mouth, the nose, the larynx (causing fatal suffocation) and even the Eustachian tube. (Eulenburg: Real Encyklopädie der Gesammten Heilkunde, 1896, X, 269, etc.)

Vomiting of tapeworms or their segments, however, is a very much rarer occurrence, and only very few such cases have been reported. Only five instances have been collected in the great work on Animal Parasites by Davaine. (Traité des Entozoaires. Paris, 2d ed.) Mosler and Peiper (Nothnagel's Handbuch, 1897, VI, 58) write: *nur äusserst selten ist der Abgang von Proglottiden beim Erbrechen gesehen worden.* My second case undoubtedly belongs to this class. That abortion should have been induced by the shock is not at all surprising, and recalls one of the cases quoted by the above writer, where a peasant was seized with a vomiting spell, and expelled part of a worm by mouth. A physician tried to pull out the rest, but the poor fellow, frightened, thought that his bowels were being drawn out and would not allow it.

In this connection I have analyzed all the cases of tapeworm and ascaris infection admitted to the Johns Hopkins Dispensary in the last eight years.

I have found in all 65 cases of tænia infection, and nine cases of infection with ascaris lumbricoides. Of the 65 tænia cases, 42 were diagnosed as tænia saginata, one was bothryocephalus latus, and in the remaining cases the species was not stated. It was interesting to note that the majority of tapeworm cases (42 out of 65) were in women, and the ages of the patients were almost all below 40 years. Vomiting as a symptom is noted only in five out of the 65 cases, or 7%, a figure rather less than that given by Stiles (Osler's Modern Medicine I, 560). Of the ascaris cases there was only one case out of the nine that gave a history of vomiting, and all of these cases with the exception of one were in children, unlike the one I described, which occurred in an adult.

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